THE PHILIPPINE
JOURNAL OF SCIENCE
B. MEDICAL SCIENCES
VOL. IV JUNE, 1909 No. 3

THE DEVELOPMENT OF A PIROPLASMA AND TRYPANOSOMA OF CATTLE IN ARTIFICIAL CULTURE MEDIA.

By ERICH MARTINI.[1]

(From the Biological Laboratory, Bureau of Science.)

Since January, 1909, I have been on leave of absence in Manila. During this time I have been employed in the Biological Laboratory where I have had the opportunity of making blood examinations in cattle. Among the dozen apparently quite healthy calves placed at my disposal, I found in one the parasite of surra, which disease is not uncommon in the Philippines; in another calf I discovered a species of piroplasma. This latter parasite reminded me both of the piroplasma of coast fever—discovered by Robert Koch(1) in Rhodesia—and of the piroplasma of cattle discovered by Dschunkowsky and Luhs(2) in Caucasas; it also resembles somewhat the piroplasma described by Miyajima and Shibayama(3) in Japan, and the one which I (4) found in Shantung and in Petschili, China. Hunter(5) in Hongkong has also given a description of a piroplasma of similar morphology; unfortunately he has published no illustrations of this parasite. Recently Schein(6) has made an observation similar to that of Hunter, in Indo-China, his article containing numerous drawings. In many points there are variations between the piroplasma I have found in Manila and the other piroplasmata mentioned; however, these variations are probably only of secondary importance. The different forms of the Manila piroplasma are illustrated

[1] Surgeon major, of the Imperial German Navy; director of the government hospital and hygienic laboratory at Tsingtau, China; at present a member of the staff of the Biological Laboratory, Bureau of Science, Manila.

in the half-schematic drawings (Plates I and II) which were made from fresh blood smears stained with Romanowsky-Giemsa solution. In the drawings, the thick, dark places are intended to represent the chromatin staining red, and the fine lines the boundaries of the parasites. Plate I, fig. 1, shows the young forms resembling those described by Koch; they are found sometimes singly and sometimes in pairs. In fig. 2 one also sees a resemblance to his ring forms. In fig. 3 there are forms with double nuclei, which frequently are found in the blood of animals infected with the other species of piroplasmata mentioned above. The cross forms described by Robert Koch as characteristic for the Coast fever group were also found in the blood of the calf infected with the Manila parasite; these are very probably composed simply of a pair of parasites with double nuclei placed side by side. (See Plate I, fig. 3, *c*, and Plate III, figs. 1 to 5.) In Plate I, fig. 4, a form is illustrated which I have not seen in the blood in infections with any other piroplasmata; it is somewhat arrow-shaped; sometimes the head of the arrow constitutes a solid chromatin mass, or forms a hollow triangle at whose apex the nucleolus lies and at whose base a larger mass of chromatin is situated. A similar form pictured in Plate I, fig. 4, *c*, at first glance might give the impression that the parasite is a small intracellular trypanosoma, an impression which would be strengthened by the appearance of an intracellular, binuclear form shown in Plate I, fig. 5, *b*, and also in Plate III, figs. 6 and 7. Finally I encountered once in the centrifugated blood a form (see Plate I, fig. 5, *c*) lying free in the blood plasma which it is advisable to describe here, on account of its relationship to the forms seen later in other calves. It was of about the size of a red blood corpuscle; its form was somewhat round and on one side the protoplasm was drawn out in a wedge shape. This irregularity of form perhaps resulted by pressure when the blood smear was made, in which case, obviously, no special weight should be laid upon the shape. The plasma of the parasite stained a bright blue; at the base of the wedge-shaped portion there are round chromatin masses stained a bright red, and on the opposite side at each of the angles is a dark brown chromatin granule. It is not clear what stage this form represents in the life cycle of the parasite; it is illustrated in Plate I, fig. 5, *c*, and in Plate III, fig. 8. It is not improbable that with special study, forms analogous to the peculiar ones described above and illustrated in Plate I, figs. 4 and 5, will be discovered in the life cycle of the other piroplasmata. These forms and the very interesting results which Mijayama obtained; namely, a growth of trypanosoma-like organisms in cultures made from the blood of cattle suffering with piroplasmosis, led me also to make blood cultures after the manner of this Japanese investigator.

For purposes of description, I shall refer in this article to the calf, in which I first saw the piroplasma, as the "original calf," in order to distinguish it from the calves used in the subsequent experiments; the

animal was a female, one year of age at the time the experiments were begun and was kept protected from ticks, flies and mosquitoes in a screened stall.

PRELIMINARY EXPERIMENTS.

On the 18th of January, 1909, 50 cubic centimeters of its blood were drawn from the jugular vein and then defibrinated, care being taken to avoid bacterial contamination of the blood. Two cubic centimeters were placed into each of several tubes containing 10 cubic centimeters of bouillon. Five of the tubes contained 1 per cent alkaline bouillon and five 1 per cent acid bouillon, phenolphthalein being used as an indicator. The tubes were placed in the incubator kept at a temperature of 26° to 27° C.

Thirty-three hours after making the cultures a trypanosoma (a division form) with a distinct flagellum was found in one of the tubes. Trypanosomata appeared later in many of the tubes, but only after a period of from forty-four to forty-eight hours. In general the parasites were about the size of the rat trypanosoma (*Trypanosoma lewisi*) and, like it, showed very considerable variation in size. There were some which measured longitudinally, together with the flagellum, one and one-half to three times the diameter of a red blood cell of the calf, and others about six to seven times this length. Occasionally, and then generally in the later days of the culture—that is, from the fifth to the sixth day—I found forms which measured from twenty to twenty-five times the diameter of the red blood cells of the calf. The smallest form was just as distinct and well developed as the largest one. The principal characteristic was a club-like swelling at the anterior (2) extremity of the flagellum, as is seen from the drawings and especially from the photographs. (Plate I, fig. 6, *b*, *c*, and *d*, and Plate III, figs. 10 to 13, and Plate IV, figs. 14 to 17.) The blepharoplast lies with its long axis perpendicular to the axis of the trypanosoma, and the nucleus parallel to this axis; the blepharoplast is usually anterior to the nucleus and is posterior to it only in exceptional cases. This trypanosoma reminds one of that found in the bison and described by Wrublewski (8) in Russia. Wrublewski's article was called to my attention after I had found the trypanosoma in my cultures.

In the cultures on acid media numerous chromatin granules appeared in the body of the trypanosomata. Both acid and alkali media seemed equally good for the cultivation of the parasites. However, for the further experiments only the acid medium was used. The motility of the parasites was similar to that of other trypanosomata which bear the flagellum at the anterior end. Only in the case of obstruction did the parasite move posteriorly.

Among the well-developed forms observed there were a few very small

[2] In this article the end of the trypanosoma upon which the flagellum is situated is considered to be the anterior one. (Co-Editor.)

ones in which the flagellum was very short and the undulating membrane poorly developed. These small forms (see Plate I, fig. 6, *a*, and Plate III, fig. 9) might suggest, on superficial examination, that they were transition forms between the trypanosoma-like organism (already described as lying in the red blood cells) and the fully developed trypanosoma. However, these rudimentary forms were always found at the same time as the well-developed ones. A chronological transition could not be observed, in spite of critical observations made every hour.

A careful study of stained preparations from the cultures showed a remarkable fact. The parasites lying within the red blood cells, that is, the piroplasmata already described, remained in the cultures until the fifth day, apparently without having increased or decreased in number: at the same time the trypanosomata showed an abundant multiplication forming large rosettes. The latter were photographed, while living, in a hanging drop preparation (see Plate V, fig. 19), in smear preparations the large masses of the parasites were torn to pieces. While the multiplication of the trypanosomata occurred as mentioned above, no development was noticed in the piroplasmata, although hourly observations were made both during the day and for a portion of the night. One noticed only a gradual swelling of the parasites and that the chromatin and protoplasm stained badly, a phenomenon which went hand in hand with the swelling of the erythrocytes. Finally on the fifth day nothing more of the piroplasmata was to be seen. Transition forms between the piroplasmata and trypanosomata were not observed either in the beginning or in the later stages of the cultures.

On about the third day of cultivation the trypanosomata were inclined to assume involution forms; that is, to become somewhat thicker and shorter and to lose their flagella. The chromatin became disintegrated and stained badly; the blepharoplast small and like a point; yet the outlines of the parasites were fairly distinct. Occasionally one had the impression that a sexual increase had set in (see Plate V, fig. 26), forms resembling spermatozoa apparently appearing in the cultures. However, a careful study with the microscope immediately dispelled such an idea, since these forms were seen to be merely involution forms of two trypanosomata and their degenerated flagella. It is important to emphasize that a development of piroplasmata into trypanosomata was, therefore, in spite of the most careful study, not observed. At the same time a careful examination of the fresh blood of the "original calf" for the presence of trypanosomata was made. Smears of the blood were made every day and on about ten occasions blood from the jugular vein was centrifugated and smears from the surface examined. The result was always the same; the piroplasmata were always present, trypanosomata never. At the same time the question of the pathological properties of the trypanosoma for monkeys was studied. Three monkeys, *Cynomolgus philippinensis* Geoffr., were given subcutaneous injections of 30 cubic centi-

meters of blood from the "original calf" and afterwards showed no signs of an infection. On the other hand, two monkeys of the same species, to which Mr. Clegg of this laboratory gave, on the 19th of January, 1909, 1 cubic centimeter and 50 cubic centimeters, respectively, of blood from the calf infected with surra, showed an enormous number of parasites in the peripheral blood, and both died, one on the 2d of February, the other on the 5th of February, 1909; the first having lived fourteen the other seventeen days after the inoculation. The subcutaneous injection of one of our cultures containing numerous trypanosomata into each of three monkeys of the same species gave likewise a negative result. In other experiments inoculations in this same species of monkey were made for the purpose of excluding the surra parasite, because it is well known in the Philippines that these monkeys are extremely susceptible to this disease. These investigations appeared to be necessary in spite of the morphological differences which are evident between the trypanosoma found in the "original calf" and the trypanosoma of surra (see Plate I, fig. 7, and Plate V, fig. 27); for the surra trypanosoma has not yet been cultivated artifically successfully and no one can know what form it will assume in the culture. I, also, have been unsuccessful in cultivating the trypanosoma of surra from the blood of cattle, horses, and monkeys. In the culture media mentioned above which is favorable for the development of the trypanosoma obtained from the "original calf," the trypanosoma of surra appears to die out quickly; this is another point of differentiation between the two. Therefore, the trypanosoma cultivated from the blood of the "original calf" is evidently not *Trypanosoma evansi,* but is a form not hitherto described.

PREPARATIONS FOR A CONTINUATION OF THE WORK.

These investigations did not decide definitely whether the trypanosomata were developed from the piroplasmata or whether the trypanosomata being extremely scarce in the blood of the "original calf" or being present in an undeveloped form simply multiplied in the culture, as certain varieties of trypanosomata are known to do. In order to solve these questions it was necessary to carry on other experiments. The artificial transmission of the infection to other calves at once suggested itself. This was undertaken, but great difficulties were immediately encountered. The pathogenicity of both the piroplasma and trypanosoma could only be determined by the inoculation of nonimmune animals. It was extremely difficult to obtain such animals, since in Luzon numerous cattle diseases appear to be rife. It was, therefore, necessary either to import cattle from districts free from piroplasmosis or, as a last resort, to use animals relatively slightly immune, that is, new-born calves. The importation of cattle was impracticable, because it was desirable to begin the inoculations at once, owing to the fact that it could not be known how long the parasites would remain in the blood of the "original

calf." It would have taken weeks and perhaps months to import the foreign cattle.

The calves of the native carabao were not used because the daily bath, which would have been necessary for them, would have consumed too much time. I chose, therefore, for the experiments the new-born calves of cows imported from Indo-China. Through the kindly assistance of Dr. Gearhart, of the Bureau of Agriculture, the Laboratory finally obtained seven of these calves. One of them died soon after its arrival from a severe phlegmon of the abdominal wall, and one was dead when it arrived at the Laboratory. Experiments were begun with the remaining five. These calves were all less than eight days old and hence only an hereditary immunity had to be considered. This I hoped to overcome, in case it existed, by inoculating very large doses of the infectious material, as I had found it possible to do in the case of rinderpest at Tsingtau, Shantung.

The calves were nourished with preserved milk which caused disturbances of nutrition and of development; these disturbances must be mentioned as they served as possible factors favoring the infection. The servants had to be especially trained to feed the animals. I omitted temperature determinations in the experiments, because I did not care to leave this to the servants and had not the time to perform the work myself. Observations on the temperature were moreover not essential, since the chief aim in view was a study of the parasites, and the clinical features of the condition were considered to be of only secondary interest and were reserved for later work. Ticks (*Boophilus australis* Fuller, as determined by Mr. Banks of this laboratory) were found on the first of these young calves. The calf was placed for ten days in quarantine to see if it would develop Texas fever; since this disease did not appear during a further period of ten days the animal was regarded as free from the infection. All the animals were placed in a division of the vaccine stable of which Dr. Ruediger was in charge; here they were protected from ticks, flies, and mosquitoes. I take this opportunity of thanking Dr. Ruediger for his cordial coöperation. Owing to the fact that the same servants attended all the animals in the stables, the calves were perhaps exposed to unknown infectious intestinal diseases, since occasionally animals used for the preparation of smallpox vaccine died; a study of these intestinal disorders was not made. I can speak of them, therefore, only in general terms. All of the five calves used in my experiments finally died of intestinal disorders, fortunately, however, after the investigations had been carried to a successful issue. The further experiments with other calves were carried on in a special stable constructed for the purpose, which was also protected from ticks, flies, and mosquitoes; separate servants were also provided for these animals and so, any possibility of intercurrent infections from other calves was excluded. At this time

older calves of cows imported from Australia had been obtained, and on these the first experiments were repeated and the previous results confirmed.

Another difficulty in continuing the work was due to the fact that during the dusty month of March it was hard to keep the blood specimens sterile; it was, therefore, often necessary in spite of the tropical heat to carry on the experiments with the calves, in the laboratory with the windows and doors closed.

It was also difficult to keep the incubators at the desired constant temperature because of the frequent changes in the atmospheric temperature rendering necessary frequent regulation of the incubators. It is desirable to mention these circumstances in order to give a correct understanding of the difficulties encountered in carrying on the work.

EXPERIMENTS WITH NEW-BORN CALVES FROM FRENCH INDO-CHINA.

In the following experiments an attempt was made to determine:

1. Whether the piroplasmosis of the "original calf" constituted a species of Coast fever or one of Texas fever.

2. Whether the trypanosomata, when isolated and injected into calves, would cause in these animals a piroplasmosis or a trypanosomiasis, or both.

3. Whether after the injection of the fresh blood of the "original calf" into other calves trypanosomata could be obtained from the animals by culture.

EXPERIMENTS.

These experiments were carried on in the following manner:

Calf No. 1 (infected with cultures).—January 26, 1909; new-born female calf, from Indo-China; ticks (*Boophilus australis*) present; nourished artificially. Blood smears examined daily for piroplasmata and trypanosomata negative. Blood culture negative. In order to exclude infection with surra, a monkey was injected with 30 cubic centimeters of the blood subcutaneously; it remained healthy and free from parasites. (In all of the experiments, the native monkeys were kept under observation for several months, and examinations of the blood were made daily).

On the 5th of February, 1909, calf No. 1 was injected with two five-day-old cultures made from blood of the "original calf," and showing a good growth of trypanosomata; at the same time a monkey was inoculated with a similar culture subcutaneously. I had been unable to find piroplasmata in these cultures on the day of the injection. The monkey remained healthy and free from parasites. On the 9th of February, 1909, the calf developed diarrhœa, but without undergoing much disturbance of its general health. On the 12th of February, 1909, the diarrhœa stopped.

On the 12th of February a culture was attempted with the blood of the calf, but gave negative results. On the 15th of February blood smears showed piroplasmata of the same morphology as those of the "original calf" and some like the *Pirosoma bigeminum*. (See Plate II, fig. 1, *a*, *c*, *e*, *f*, *g*, *h*, and Plate V, figs.

20, 21, and 24.) On the 16th of February an attempt at culture of the blood of this calf was again made, but contamination with bacteria occurred.

On the 17th of February another attempt at cultivation of the parasite was made which resulted positively. On the 19th of February trypanosomata were found in this culture. Diarrhœa began again on the 18th of February and led to a rapid loss of strength and finally to the death of the calf on the 22d of February. The death of the animal was probably due to stall infection through carelessness of the stable boy, or to unfavorable conditions produced by artificial feeding.

Autopsy.—Marked emaciation, discharge from eyes and nostrils, lungs hyperæmic. The mesenteric glands were hæmorrhagic and swollen to about the size of a small bean. Catarrh of the large and small intestines was present. Otherwise no pathological changes of importance were noted.

The plasma bodies regarded by Koch as characteristic for Coast fever were not found in smears from either the spleen or lymph glands.

This experiment showed—

1. The presence of an infection which belongs in the Texas fever group. An infection with a variety of Coast fever did not enter into the question, because the piroplasmata of this disease are not transferable by a single blood inoculation, but only by repeated ones (Koch).

2. The possibility of transferring the trypanosoma to animals by the inoculation of cultures. The question of whether these trypanosomata had developed from the piroplasmata remained unanswered; for the culture succeeded only after the piroplasmata had been found in the blood; on the other hand, the piroplasmata could be recognized in the cultures up to the fifth day, during which time the trypanosomata had shown a marked increase; stages of these parasites which might have been regarded as transition forms from the piroplasmata were never seen.

For fear that this animal (upon which ticks were discovered at the beginning of the experiment) might have been infected with Texas fever in spite of the precautions taken, it seemed necessary to repeat the same experiment with another calf (see calf No. 3, below).

Calf No. 2 (infected with fresh blood).—February 5, 1909; new-born calf from Indo-China, female, free from ticks; at first fed with mother's milk, later after the death of the mother, fed artificially with preserved milk. Blood smear free from piroplasmata and trypanosomata. Blood culture negative. A monkey was given 30 cubic centimeters of its blood subcutaneously and remained healthy and free from parasites.

On the 5th of February the calf was injected subcutaneously with 30 cubic centimeters of blood from the "original calf."

On the 18th of February the first piroplasmata appeared in the blood and were of the same appearance as those observed in the new-born calf No. 1. (See also Plate II, fig. 1, *b* and *d*, and Plate V, figs. 23 and 25.)

On the 19th of February the mother of the calf died with symptoms of rinderpest. Artificial feeding was begun. On the 21st of February diarrhœa began, leading to a rapid loss of strength and death of the calf on the 23d of February. Cause of death probably the same as of calf No. 1.

Autopsy.—Marked emaciation. Discharge from eyes and nostrils; mesenteric glands hæmorrhagic and swollen to the size of a pea or small bean. Catarrh of

the large and small intestines present. Liver shows slight icterus. Otherwise no change noted. The plasma bodies of Koch were not found in smears from either the spleen or lymph glands.

The attempt to cultivate trypanosomata from the blood of this calf resulted negatively, although the culture medium showed no contamination with bacteria, which might have hindered the development of the trypanosomata.

The experiment with calf No. 2 showed—

1. The existence of a variety of Texas fever as in calf No. 1; infection with Coast fever was not considered for the reasons already mentioned in the experiment with calf No. 1.

2. The failure in this instance to transfer trypanosomata by means of the fresh blood of the "original calf."

Therefore, the results of the experiments on the first two calves showed a striking contrast. Hence the question arose whether the piroplasmata, which I had, to be sure, not found on the fifth day of culture when I injected the cultures of the blood of the "original calf" into calf No. 1 on the 5th of February, had nevertheless been present in the culture in a condition able to cause infection, and whether they were not transferred together with the trypanosomata and succeeded in developing in calf No. 1.

An experiment with another calf (No. 3) was planned to settle this question.

Calf No. 3 (repetition of the experiment on calf No. 1).—February 14, 1909; new-born female calf from Indo-China; free from ticks; fed artificially. A daily examination of the blood for parasites was made with negative results. A culture from the blood remained sterile. A monkey was injected with 30 cubic centimeters of the blood and remained healthy and free from parasites. The calf received subcutaneously on the 24th of February two seven-day-old cultures from the blood of the "original calf." These cultures showed a good growth of trypanosomata, but piroplasmata could no longer be found in them. At the same time a monkey was given subcutaneously a similar seven-day-old culture. The monkey remained free from parasites, but died on the 2d of April, 1909, from an unknown cause. A blood specimen examined shortly after its death revealed no parasites.

The calf became sick on the 27th of February with diarrhœa, rapidly lost strength, and died on the 3d of March. The cause of death was the same as in calves Nos. 1 and 2.

Autopsy.—Marked emaciation, catarrh of the large and small intestines; mesenteric glands the size of a pea or a small bean. Otherwise no pathological changes were noted. The plasma bodies of Koch were not found in smears from the spleen and lymph glands.

A blood culture made on the 3d of March shortly before the death of the calf developed a growth of trypanosomata, while piroplasmata were never found in spite of repeated daily examination of the blood.

This experiment resulted in the cultivation of trypanosomata in the absence of piroplasmata.

Seven-day-old cultures were chosen for producing the infection, because it was hoped that the piroplasmata would have died out in the cultures during this time, it already having been shown that the trypanosomata live longer than this. Nevertheless the results of this experiment might have been accidental, since a piroplasmosis in this calf might have existed and escaped observation. This point deserves especial consideration, since Miyajima(7) (page 90) in performing similar experiments observed on one occasion that in a calf inoculated with a trypanosoma culture, after three days developed trypanosomata in its blood as was proved by obtaining cultures of trypanosomata from it, while piroplasmata were only found seven days later than this.

Therefore, it was planned to repeat this experiment with piroplasmata which had been subjected to conditions much more unfavorable to their life and development.

Calf No. 4 (repetition of the experiments carried on with calf No. 2).—February 26; new-born male calf from Indo-China; free from ticks; fed artificially; blood free from parasites; blood culture negative. A monkey was given 30 cubic centimeters of blood subcutaneously and remained healthy and free from parasites.

On the 26th of February calf No. 4 was inoculated with 30 cubic centimeters of blood of the "original calf" subcutaneously.

On the 5th of March piroplasmata, of the same appearance as those encountered in calves Nos. 1 and 2, were found present.

On the 7th of March a bloody stool was passed. An examination of the peripheral blood showed numerous red blood cells infected with piroplasmata.

On the 10th of March a discharge from the eyes and nostrils appeared. Diarrhœa was present and the animal appeared to lose strength rapidly.

On the 13th of March after the blood had been taken again for culture the calf died. The cause of death was probably the same as in calves Nos. 1, 2, and 3.

Autopsy.—Marked emaciation, discharge from eyes and nostrils; mesenteric lymph glands hæmorrhagic and swollen to the size of a pea or small bean; catarrh of the large and small intestines; liver sligthly icteric. Otherwise no pathological changes observed. The plasma bodies of Koch were not found in smears either from the spleen or from the lymph glands.

A blood culture made on the 5th of March showed no development of protozoa, probably on account of contamination with bacteria.

Attempts at cultivation on the 9th, 10th, and 11th of March showed the presence of forms which I had not encountered either in cultures from the "original calf" or in those from calves Nos. 1 to 3. In these cultures from calf No. 4 the piroplasmata showed no development into trypanosomata, but on the other hand forms were found to be present which Robert Koch(1,*c*) had encountered in ticks and described as the first stages of development of *Piroplasma bigeminum.* (See Plate II, fig. 2, *a, b, c,* and Plate VI, figs. 30 and 31.)

On the first day of cultivation the piroplasmata, which showed two distinct chromatin masses, were found to have become free from the red blood cells which they had severely injured. The injury to the red

blood cells could be recognized by the irregular form which they assumed and by the marked metachromasia present. The piroplasmata assumed a rounder shape and became collected in larger groups. A number of them showed the characteristic rays first described by Robert Koch, which are probably composed of protoplasm, since with the Romonowsky-Giemsa stain they acquire the same blue color as the remaining protoplasm. These ray forms appeared to be especially distinct on the second or third day of cultivation. The piroplasmata in the culture became larger and showed as a rule only two chromatin masses in the blue ground of their protoplasm. Later the forms of Koch with chromatin points appear; usually they are found on the third day for the first time.

These forms recall those resembling an arrow point which were found within the red blood cells of the "original calf." There is a temptation to assume a relationship between them. (See Plate I, fig. 4, *a*, and Plate II, fig. 2, *d*, and Plate VI, fig. 32.)

Beside these forms there were still others observed in the cultures which were of about the size of a red blood cell and which contained from two to three bright red chromatin masses and several (2 to 4) dark brown red chromatin granules. Their form was in general oval; many of them showed rays which stained the same bright blue color as the remaining protoplasm. These large forms were found scattered among the smaller ray forms and occasionally were situated apart from them. The significance of these forms is not clear. In morphology they conform so closely to those observed outside the red blood cells which were found in the fresh blood of the "original calf," that one must think of a relationship between the two (see Plate II, fig. 2, *e*, and Plate VI, figs. 33 and 34; and Plate I, fig. 5, *c*, and Plate III, fig. 8.)

One might be inclined to regard them as macrogametes; yet there is need of further proof before such an opinion is justified. Also the idea that the forms with chromatin points which appear at the same time are microgametes requires confirmation. Further stages of development have not been observed. All these forms described above apparently died after from five to about eight days in the culture media kept at a temperature of 28° to 29° C.

This experiment showed—

1. The presence of a variety of Texas fever organism in the blood of calf No. 5 as in that of calves Nos. 1 and 2.

2. The failure to transfer trypanosomata by means of the inoculation of the fresh blood of the "original calf," as was the case with calf No. 2.

Calf No. 5 (repetition of the experiment performed on calves Nos. 1 and 3).—March 4, 1909; new-born male calf from Indo-China; free from ticks; fed with preserved milk; blood smears show no parasites; blood culture negative. A monkey received 15 cubic centimeters of its blood subcutaneously and remained healthy and free from parasites.

On the 4th of March the calf was injected subcutaneously with a 28-day-old culture containing trypanosomata which were feebly motile and in which piroplasmata were no longer found; other cultures were unfortunately not at hand.

On the 13th of March an attempt at cultivation from the blood of the calf was unsuccessful, although no contamination of the culture media with bacteria resulted.

On March 16 the attempt at cultivation was again unsuccessful, although there was no contamination of the culture media with bacteria.

March 17: Diarrhœa began which led to a rapid loss of strength and death of the calf on the 18th of March. The cause of death was probably the same as in the cases of calves Nos. 1 to 4.

Autopsy.—Marked emaciation; mesenteric glands the size of a pea to a bean; catarrh of the large and small intestines. Otherwise no pathological changes noted. The plasma bodies of Koch were not found in smears either from the spleen or from the lymph glands.

This experiment showed—

The possibility of the failure of an attempt at infection of a calf with the trypanosomata, from the injection either of too small an amount of the culture, or of too old a culture, that is, of one which is weakened in its virulence. The early death of the calf leads one to assume that this conclusion is only conditionally correct, since, if the animal had lived longer, further attempts at culture might have shown finally, the presence of an infection with trypanosomata. Nevertheless the experiment was valuable as indicating in future experiments the inadvisability of employing for inoculation the contents of a single culture tube or that of one so old.

EXPERIMENTS WITH CALVES FROM INDO-CHINA AND AUSTRALIA.

The experiments with the following calves were made in a new stable protected against rinderpest and other infection that could be acquired by contact. The calves were well beyond the first days of life. No. 6 was a calf from Indo-China not quite a month old; Nos. 7, 8, and 9 were Australian calves four to five months old. They were all free from ticks. Calves Nos. 6, 7, and 8 were fed with preserved milk and dry food, calf No. 9 with dry food alone.

Calf No. 6 (repetition of the experiments with calves Nos. 2 and 4).—March 26, 1909; a male calf from Indo-China; free from ticks; blood smears free from parasites; blood culture negative. Smears made from the surface of the centrifugated, defibrinated blood were free from parasites. Thirty cubic centimeters of its blood were injected subcutaneously into a monkey; the monkey remained healthy and free from parasites.

March 26: The calf received subcutaneously 50 cubic centimeters of the blood of the "original calf."

April 3: Piroplasmata found in blood smears.

On April 3, 6, 10, and 14 and May 4 attempts at blood cultures were negative, as regarded trypanosomata, although there was no contamination with bacteria.

Piroplasmata like those seen in the other calves were found in the cultures until the 24th of April. They were usually scarce. A change

of the piroplasmata into the developmental forms of Koch as seen in the cultures from the blood of calf No. 4 was not observed. On account of the scarcity of the parasites these forms were not specially searched for, since, according to the experience with calf No. 4, a multiplication of the piroplasmata in these cultures was not to be expected.

The calf became thin, but suffered in general only slightly from the infection as is the custom with calves inoculated with virulent blood from Texas fever cases.

It was killed on May 12 and no pathological lesions were found in the internal organs; no plasma bodies of Koch were seen in smears from the spleen and lymph glands.

Calf No. 7 (repetition of the experiments on calves Nos. 2, 4, and 6).—March 26; female Australian calf about 4 months old; free from ticks; blood culture negative; smears from the surface of the centrifugated defibrinated blood, free from parasites. Thirty cubic centimeters of the blood of the calf were injected subcutaneously into a monkey, which remained healthy and free from parasites.

March 26: The calf received subcutaneously 50 cubic centimeters of blood from the "original calf."

April 1: Piroplasmata found present in blood smears. They remained numerous until the 5th of April, and from that time were present only in small numbers.

On April 1, 2, 3, 14, and 30 blood cultures were made which were *negative in regard to trypanosomata, but positive with regard to the developmental forms of the piroplasmata described by Koch.* (See Plate VI, figs. 28 and 29.)

April 3: Animal passed a very hard stool with streaks of blood.

April 30: Examination of the blood showed that almost all the piroplasmata revealed the morphological characteristics of those observed in the "original calf"; the *Piroplasma bigeminum* form was rare.

The calf suffered but little from the infection. Only a moderate anæmia was present.

It was killed on May 12. The autopsy showed a slight icterus of the liver, which was the only pathological change observed. No plasma bodies of Koch were found in smears from the spleen or lymph glands.

The results of the experiments on calves Nos. 6 and 7 confirm those obtained with calves Nos. 2 and 4.

They show—

1. The presence of an infection belonging to the Texas fever group.

2. The failure to transfer trypanosomata by means of an injection of the fresh blood of the "original calf."

After experiments on calves Nos. 2, 4, 6, and 7 had resulted as described above it only remained to confirm the results of the experiments on calves Nos. 1, 3, and 5. The experiment on calf No. 3 had indicated that by using a trypanosoma to culture seven days old the pirosomata contained therein could be excluded so far as the production of an infection with them was concerned. Therefore, it was possible that the

trypanosomata alone and without the piroplasmata had caused infection. Experiment with calf No. 5 seemed to demonstrate that either the number of trypanosomata in a single culture tube might not be sufficient to produce an infection or that the culture, which was twenty-eight days old, had become so attenuated that not only the pirosomata, but also the trypanosomata had been deprived of their power of causing infection. The object of the following experiment was to kill the piroplasmata and at the same time to preserved the trypanosomata alive in the culture.

Calf No. 8 (repetition of experiments on calves Nos. 1, 3, and 5).—April 3, 1909; female Australian calf about 4 months old; free from ticks; blood smears free from parasites; blood culture negative; smear from the surface of the centrifugated blood free from parasites. The inoculation of two monkeys with 20 and 50 centimeters respectively of the blood of the calf gave negative results.

April 12: After the blood of the calf had been found to be free from parasites by daily examination of simple blood smears and smears from the surface of the centrifugated blood, the calf was given subcutaneously three five-day-old trypanosoma cultures obtained from the blood of the "original calf." The culture had been grown at a temperature of 29° to 31° C. A monkey was given a similar culture subcutaneously. Piroplasmata could not be found in these cultures. Cultures grown at this high temperature were chosen because under such circumstances it was anticipated that the piroplasmata would die out, while it had already been determined that the trypanosomata remained alive at this temperature. Both the calf and the monkey remained healthy and lively. Daily examinations of simple blood smears and repeated examinations of the centrifugated fresh blood gave negative results.

On April 21 and on May 5 trypanosomata were cultivated from its blood, but they could not be shown to be present in any other way. In spite of most careful examinations piroplasmata were never found up to the thirty-seventh day after the infection.

On May 17, the thirty-seventh day after the infection, the calf was killed. The autopsy showed that the organs were normal. The plasma bodies of Koch were not present in smears from either the spleen or lymph glands.

Calf No. 9 (repetition of the experiments on calves Nos. 1, 3, 5, and 8).—This experiment was planned in case experiment No. 8 should have been unsuccessful.

April 16: Australian male calf, 4 months old, free from ticks; blood smears and smears from the surface of the centrifugated blood showed no parasites; blood culture negative. A monkey was inoculated with 30 cubic centimeters of the calf's blood but it remained well and no parasites appeared in its blood.

On April 17 the calf received four ten-day-old cultures of trypanosomata made from the blood of the "original calf." These cultures had been grown at a temperature of from 29° to 31° C. and no longer contained piroplasmata. The calf remained healthy and lively. Daily examinations of blood smears and repeated examination of centrifugated fresh blood gave negative results; neither piroplasmata nor trypanosomata were found. *However, trypanosomata were cultivated from its blood*

on April 28 and May 5. Piroplasmata were still absent on May 17, the thirty-second day after infection with the cultures.

The calf was killed on May 17. The autopsy showed hæmorrhagic lymph glands which were somewhat enlarged. Otherwise no pathological changes were noted. The plasma bodies of Koch were not found in smears from the spleen or from the lymph glands.

The experiments on calves Nos. 8 and 9 show that it is possible to *separate the trypanosoma and the piroplasma,* the parasites infecting the "original calf." The importance of this result is the same, whether it was accomplished by the fact that both of the calves were immune to the piroplasma or, as I believe, by the killing of the piroplasma through keeping the culture for a long time at a temperature of 29° to 31° C., whereby the capability of the trypanosoma to cause infection was not destroyed.

FURTHER HISTORY OF THE "ORIGINAL CALF."

The "original calf" remained in good health and its blood contained continuously the piroplasma described above; the trypanosoma was cultivated repeatedly from its blood, for the last time on April 7. At this time piroplasmata were also still present, but trypanosomata could not be found by microscopical examination even in centrifugated blood. On May 19 while still in the best of health the calf was killed. The autopsy showed numerous hæmorrhagic lymph glands which were swollen to the size of a pea or bean; this was particularly noted in the mesenteric glands. The spleen was enlarged and somewhat soft. Otherwise no pathological changes were observed. The plasma bodies of Koch were not found in smears either from the spleen or from the lymph glands. An infection with Coast fever could, therefore, be excluded. A mild infection with Texas fever, or a variety of this disease had undoubtedly existed.

RESULTS OF ALL THE EXPERIMENTS.

From all the experiments we may conclude:

1. The "original calf" was infected with a piroplasma belonging to the Texas fever group.

2. It was also infected with a trypanosoma hitherto unknown.

3. No evidence was obtained of a transition in the blood cultures of the piroplasmata into trypanosomata or, on the other hand, of a transition of the trypanosomata, in the blood of the animal infected with them, into piroplasmata.

4. The infection with piroplasmata of calves Nos. 2, 4, 6, and 7 rendered calves Nos. 6 and 7 slightly sick, while calves Nos. 2 and 4 died of another disease, before sufficient time had elapsed for the symptoms of piroplasmosis to develop.

5. Calves which have been inoculated with the trypanosoma apparently

merely harbor this parasite and are not rendered visibly sick by infection with it.

The following questions remained to be answered:

1. Why did I not succeed in obtaining in culture media the developmental forms of Koch from the piroplasmata of the "original calf"?

2. Why did I not succeed in infecting with trypanosomata calves Nos. 2, 4, 6, and 7 by means of the direct inoculation of the fresh blood of the "original calf" without the use of culture media?

In relation to the first question, it may be stated that a growth from the forms in the blood of the "original calf" into well-developed piroplasmata probably took place; however, these well-developed forms of Koch were probably very rare as compared with the number of rod-shaped and similar ones present in the blood (owing to the animal being relatively immune though carrying the parasites) and for this reason they escaped observation. Apparently this was also true in the case of calves Nos. 2 and 4. The piroplasmata do not multiply typically in the culture media, but only undergo a simple change in morphology, the meaning of which is at the present time not fully known to us. In spite of the most careful study I have never seen the parasites having rod-like and similar forms develop into those which Koch described; the former always appear to disintegrate. Furthermore, it might be mentioned here that I have not observed among the forms of the Manila piroplasma the amœba-like cells and those containing vacuoles which Miyajima describes as being stages in the development of his piroplasma. I have likewise not noticed the increase in size or the differentiation, in the ring forms mentioned by him, so that such forms are not stages in the development of the piroplasma I have observed. I believe, however, that I have seen somewhat similar forms in incubated mixtures of normal fresh blood of cattle and bouillon. When the piroplasmata were present in enormous numbers, as for example, in calves Nos. 4 and 7, I have seen them develop in the culture media into the ray forms of Koch and to the forms with chromatin points. However, when they were present in smaller number, the developmental forms simply escaped observation. If there had been an abundance of time which I had not needed for more important work, I am convinced that I could have found these forms also in such instances.

The second question with regard to the failure to infect with trypanosomata the calves Nos. 2, 4, 6, and 7 by means of the inoculation of fresh blood of the "original calf" can also be answered. The fact that, in spite of the inoculation of cultures containing enormous numbers of trypanosomata (as in the experiments with calves Nos. 1, 3, 5, 8, and 9), I could not find trypanosomata in their fresh blood, even after centrifugation, shows that the trypanosomata have undergone only a limited multiplication in these animals which, therefore, must have a high degree of natural immunity against the trypanosomata. This is shown even

more strikingly by the fact that as a rule only a third of the ten to twenty cultures from the calves showed trypanosomata; in fact, not infrequently trypanosomata were found in only a single tube. Hence they must have been present in extremely small numbers in the blood of the infected calves. This fact is in full accord with my experiences with a strain of *Trypanosoma brucei* of low virulence with which I(10) worked for years under the direction of Robert Koch. I succeeded in infecting other animals with the strain, but in the blood of the original animal itself, a mare from Togo, I did not find trypanosomata in spite of observations lasting for months. I am inclined to believe that the trypanosoma described in the present experiments would appear in greater number in the fresh blood of a species of animal which is especially susceptible to it could one be found. Dogs which were given very large doses of the blood of the Togo mare, subcutaneously or intraperitoneally, after a certain period of incubation, showed the tsetse trypanosomata in their blood. The failure to find trypanosomata in the fresh blood of the "original calf" and in the blood of the calves inoculated with cultures of this trypanosoma can hence be explained by the extreme scarcity of the trypanosomata. However, it is possible that the parasites are present in an especial form, but not that of the piroplasmata described.

CONCLUSIONS.

The experiments justify the following conclusions:

1. In the Philippine Islands there are domestic cattle apparently quite healthy which are carriers of the parasite of surra. These cattle are a continual danger, especially for horses in which surra always runs a fatal course, as has been shown by the works of Smith and Kinyoun(11), Curry(12), Salomon and Stiles(13), Strong(14), Musgrave, Williamson and Clegg(15, 16).

2. In the Philippine Islands there exists a variety of Texas fever which is perhaps identical with that seen by Jobling and Woolley(17) in the years 1903 and 1904. However, this can not be stated with certainty to-day, because these investigators gave no accurate description of their parasites. The piroplasma is probably of Indo-Chinese origin. It is a variety of *Piroplasma bigeminum* and shows in certain culture media developmental forms which correspond to those described by Robert Koch in the tick for *Piroplasma bigeminum.* Forms similar to those seen in the early stages of the development of *Piroplasma bigeminum* by Koch and to those of the Manila piroplasma have been found in artificial media by Kleine(18) for *Piroplasma canis,* and by Marzinowsky(19) for *Piroplasma equi.* Besides the early forms of Koch, the Manila parasite shows other forms with a different morphology.

3. This piroplasma, when kept in the bouillon employed and at a temperature of 29° to 31° C., dies within five to ten days.

4. In the Philippine Islands there occurs a trypanosoma of cattle which is different morphologically and biologically from *Trypanosoma evansi* and from the other trypanosomata of mammals. Its virulence for the Indo-Chinese and Australian calves appears to be slight.

5. This trypanosoma could not be discovered in the fresh blood by microscopical examination, but could be cultivated in a mixture of blood and bouillon, and could be transferred to healthy calves by the subcutaneous injection of the cultures.

6. The trypanosoma remains alive and capable of causing infection for at least ten days in the bouillon employed when kept at a temperature of 29° to 31° C. Since the piroplasma which was present at the same time in the blood of the "original calf" died out at this temperature within the above-mentioned time, it was possible to isolate the trypanosoma and to transfer it, thus separated from the piroplasma, to calves (Nos. 8 and 9).

7. These culture experiments prove the great importance of this method for the differentiation of blood parasites. The absence of the plasma bodies of Koch, the presence alone at autopsy of an icterus of the liver, and the possibility of transmitting the infection to another animal by a single inoculation of the blood, all indicated that a variety of Texas fever and not one of Coast fever was present in the "original calf." I was further confirmed in this belief by finding in the cultures made from the blood of the "original calf," developmental forms like those of *Piroplasma bigeminum* which Koch discovered in ticks. Therefore, further proof was given that a variety of the Texas fever parasite, *Pirosoma bigeminum*, and not the Coast fever parasite, was present.

8. The value of the culture experiments may also be seen by the fact that through them alone was it possible to demonstrate the presence of a trypanosoma in the blood. This to-day is of especial importance in the search for carriers of protozoa and in the study of the numerous drugs employed in the treatment of the various forms of trypanosomiasis, and especially of sleeping sickness. While the inoculation of animals can give a positive result only when the trypanosomata are virulent for the species of animal used, one can employ the method of artificial cultivation without regard to the virulence of the trypanosoma and thus can obtain results which are perhaps impossible by animal inoculation, or which at any rate, may involve much work and expense.

The cultivation of protozoa has in general an advantage over the cultivation of bacteria, since for the identification of a bacterium usually a tedious biological differentiation is necessary, while in the case of a protozoön the morphology in the culture alone often suffices to identify it.

For all these reasons careful attempts in the cultivation of these protozoa after the manner of Novy-McNeal(20), Rogers(21), Miyajima(7) and Nicolle(22) should be made until finally a simple and sure method of culture has been discovered for all pathogenic trypanosomata.

LITERATURE.

(1) Robert Koch. (*a*) Interim report on Rhodesian red-water or African Coast fever. Salisbury, Argus Printing and Publishing Company (1903).

(*b*) Second report on African Coast fever. Argus Printing and Publishing Company (1903).

(*c*) Vorläufige Mitteilungen über die Ergebnisse einer Forschungsreise nach Ostafrika. *Deutsch. med. Wchnschr.* (1905), **31**, 1865.

(*d*) Beiträge zur Entwicklungsgeschichte der Piroplasmen. *Ztschr. f. Hyg. u. Infektionskrankh.* (1906), **54**, 1; Plate 1, Figs. 15 and 16.

(2) Dschunkowsky and Luhs. Die Piroplasmosen der Rinder. *Centralbl. f. Bakt. etc.* (1904), **35**, 486.

(3) Miyajima and Shibayama. Ueber das in Japan beobachtete Rinderpiroplasma. *Ztschr. f. Hyg. u. Infektionskrankh.* (1906), **54**, 189.

(4) Martini. (a) Ueber ein Rinderpiroplasma der Proviñz Schantung (China). *Arch. f. Schiffs- u. Trop.-Hyg.* (1907), **11**, 507.

(b) Ueber das Vorkommen eines Rinderpiroplasmas in der Provinz Petschili (China). *Ibid.*, 718.

(c) Ueber die Rinderzecken Schantungs und ihre Beziehungen zu den dortigen Piroplasmosen. *Ibid.*, 740.

(5) Hunter. Annual sanitary report of the colony of Hongkong for the years 1903 and 1904.

(6) Schein. Observations sur la piroplasmose des bovidés d'Indo-Chine et constatations de piroplasmose chez les buffles. *Ann. Inst. Pasteur* (1908), **22**, 1004.

(7) Miyajima. On the cultivation of a bovine Piroplasma. A preliminary communication. *This Journal, Sec. B.* (1907), **2**, 85.

(8) Wrublewski. Ein Trypanosoma des Wisent von Bielowesch. *Centralbl. f. Bakt. etc.* (1908), **48**, 162.

(9) Banks, Ch. S. The Australian tick (*Boophilus australis* Fuller) in the Philippine Islands. *Pub. Bu. Gov't. Lab.*, Manila (1903–04), **3**, 13.

(10) Martini. Untersuchungen über die Tsetsekrankheit swecks Immunisierung von Haustieren. Unter Leitung von Robert Koch. *Ztschr. f. Hyg. u. Infektionskrankh.* (1905), **50**, 1.

(11) Smith and Kinyoun. A preliminary report on parasitic diseases of horses. Army pathological Laboratory, Manila (1901).

A preliminary report on parasitic diseases of horses. Reprint, with additions by Smith. General Orders, No. 390, Headquarters Division of Philippines. December 11 (1901).

(12) Curry, J. J. Report on a parasitic disease in horses, mules, and carabaos of the Philippine Islands. From report to the Surgeon-General, U. S. Army. December 17 (1901); *Am. Med.* (1902), **3**, 512 to 513.

"Surra" or nagana. *Ibid.*, **4**, 95.

Report on a parasitic disease in horses, mules, and carabaos in the Philippine Islands. *Vet. Journ.* (1902), **54**, 292.

(13) Salmon and Stiles. Emergency report on Surra. Bull. No. 42, Bureau of Animal Industry. U. S. Dept. of Agri. (1902), 1 to 130.

Ann. report of Bureau of Animal Industry, U. S. Dept. of Agri. (1901), 41 to 182.

(14) Strong, R. P. Animal diseases, Surra. Ann. report of the Biological Laboratory ending Aug. 31. (1902), **1**, 578.

(15) Musgrave and Williamson. A preliminary report on Trypanosomiasis of horses in the Philippine Islands. *Pub. Bu. Gov't. Lab.*, Manila (1903), **3**.

(16) Musgrave and Clegg. Trypanosoma and Trypanosomiasis, with special reference to Surra in the Philippine Islands. *Ibid.*, **5**.

(17) Jobling and Woolley. Texas fever in the Philippine Islands and the Far East. *Ibid.* (1904), **14**, 5.

(18) Kleine. Kultivierungsversuch der Hundepiroplasmen. *Ztschr. f. Hyg. u. Infektionskrankh.* (1906), **54**, 10.

(19) Marzinowsky. Ueber die Züchtung von *Piroplasma equi. Ibid.* (1909), **62**, 417.

(20) Novy and McNeal. Contributions to medical researches. June (1903), 549 to 577.

On the cultivation of *Trypanosoma brucei. Journ. Infect. Dis.* (1904), **1**, 1 to 30.

(21) Rogers, L. Preliminary note on the development of Trypanosoma in cultures of the Cunningham-Leishman-Donovan bodies of cachexial fever and kala-azar. *Lancet* (1904), **2**, 215. *Journ. Trop. Med.* (1904), **7**, 259. *Brit. Med. Journ.* (1904), **1**, 1249 to 1251. *Ibid.*, **2**, 645 to 650. *Lancet* (1905), **1**, 1484.

(22) Nicolle, Ch. Culture du parasite du bouton d'Orient, présentée par M. Laveran. *Compt. rend. Acad. Sci.* (1908), **146**, 842.

(23) v. Prowazek. Studien über Säugetiertrypanosomen. Arbeiten aus dem Kaiserlichen Gesundheitsamt. (1905), **22**, 351; Fig. 37.

ILLUSTRATIONS.

PLATE I.

Schematic drawings from preparations stained with Giemsa's solution. Magnification about 1,500 diameters.

FIGS. 1 to 5. The piroplasmata in fresh blood smears from the "original calf."

FIG. 5, *c*. Form seen in the centrifugated blood. It is not clear what stage in the life cycle of the parasite this form represents.

FIG. 6. The trypanosomata in a bouillon culture of the blood.

FIG. 7. *Trypanosoma evansi* from a smear of the fresh blood of the calf infected with surra.

PLATE II.

Schematic drawings of preparations stained with Giemsa's solution of the first pirosomata observed in calves Nos. 1 and 2. Magnification about 1,500 diameters.

FIG. 1. *a*, *c*, *e*, *f*, *g*, and *h* from blood of the calf No. 1; *b* and *d* from blood of calf No. 2.

a. A pirosoma which apparently is endeavoring to enter the red blood cell with its pointed end situated anteriorly; this form was repeatedly observed in stained preparations.

b. *Pirosoma bigeminum*, which apparently has entered recently an enlarged metachromatic erythrocyte and which has drawn after it the connecting band of protoplasm. Probably on account of the increased resistance in the red blood cell the nucleolus has moved to one side and the nucleus to the pointed end. This form was also occasionally observed in calf No. 2.

c, *d*, *e*, *f*, *g*, and *h*. These forms require no explanation. The red blood cells which contain parasites at this stage in their development are distinctly matachromatic; their shape is no longer round and in many it is irregular.

FIG. 2. The piroplasmata in artificial culture media, lying outside of the red blood cells; from calf No. 4. Magnification about 2,000 diameters.

a. Piroplasma; the earliest free form observed in the blood bouillon; compare with Koch's illustrations: Beiträge zur Entwickelungsgeschichte der Piroplasmen. *Ztschr. f. Hyg. u. Infektionskrankh* (1906), **54**, Table I, figs. 6 and 7.

b and *c*. Ray forms; compare also with Koch's illustrations; reference as above, Table I, figs. 9 to 14.

d. Forms with chromatin points; compare with Koch's illustrations; reference as above, Table I.

e. Round form with chromatin points.

PLATES III TO VI.

Magnification in figs. 1 to 17 and 20 to 34, 1,000 diameters. Magnification in fig. 18, 1,250 diameters, in fig. 19, 450 diameters. The photographs were made by Mr. Charles Martin, photographer of the Bureau of Science. Preparations stained by Giemsa's solution.

PLATE III.

FIG. 1. Narrow forms of the piroplasmata in fresh blood smears from the "original calf" made on January 18, 1909.

FIG. 2. Ring form in fresh blood smear from the "original calf" made on January 18, 1909.

FIG. 3. Binuclear form in a fresh blood smear from the "original calf" made on January 27, 1909.

FIG. 4. Double binuclear form from a fresh blood smear from the "original calf" made on February 16, 1909.

FIG. 5. Cross form from a fresh blood smear from the "original calf" made on January 18, 1909.

FIG. 6. Arrow form from a fresh blood smear from the "original calf" made on February 1, 1909.

FIG. 7. Form resembling a trypanosoma from a fresh blood smear from the "original calf" made on February 1, 1909.

FIG. 8. A form lying outside of the red blood cell in the fresh centrifugated blood from the "original calf."

FIG. 9. Rare form of trypanosoma from the bouillon culture from the "original calf" made on January 23, 1909, seen on January 25, 1909. To the left, below is seen an indication of the undulatory membrane; at the left a slight club-like swelling indicates the flagellum.

FIG. 10. Division form of a trypanosoma from the blood culture from the "original calf" made on February 5, 1909, seen on February 8, 1909.

FIG. 11. A small, well-developed trypanosoma from the bouillon culture of the "original calf" made on January 18, 1909, seen on January 20, 1909.

FIG. 12. A large well-developed trypanosoma from the blood culture of the "original calf" made on January 23, 1909, seen on January 25, 1909.

FIG. 13. Very slender trypanosoma prepared the same day from the same culture as in fig. 12. Perhaps a male individual; compare with Prowazek's illustrations (Studiem über Säugetiertrypanosomen(23)). Fig. 37.

PLATE IV.

FIG. 14. Form showing beginning division; prepared from the same culture on the same day as fig. 12. The blepharoblast shows a contraction in the middle, and the nucleus is drawn out in the direction of the long diameter of the parasite. In one of the red blood cells is seem a persisting piroplasma.

FIG. 15. Continuation of the process of division; two flagella are now visible; preparation made the same day and from the same culture as fig. 14.

FIG. 16. Continuation of the process of division, made on February 5, 1909. Daughter cells, on the point of separating; from a bouillon culture of the "original calf" seen on February 8, 1909.

FIGS. 17 and 18. Groups of trypanosomata from the same culture made on January 23, 1909, seen on January 25, 1909. In fig. 18 the magnification is about 1,250 diameters.

PLATE V.

FIG. 19. Culture of trypanosomata photographed alive in a hanging drop preparation; from a bouillon culture of the "original calf" made on January 18, 1909, photographed on January 23, 1909. Magnification about 450 diameters.

FIG. 20. Form like *Pirosoma bigeminum* in the fresh blood of calf No. 1. (Free form.)

FIG. 21. Intracellular forms observed on the same day as the forms illustrated in fig. 20.

FIG. 22. Intracellular forms observed on February 20, 1909, preparation from the fresh blood of calf No. 1.

FIG. 23. A form resembling *Pirosoma bigeminum* from the fresh blood of calf No. 2. (Free form.)

FIG. 24. Intracellular form resembling a trypanosoma found in the fresh blood of calf No. 1.

FIG. 25. Intracellular form resembling a trypanosoma found in the fresh blood of calf No. 2.

FIG. 26. Involution forms of trypanosomata found on the fifth day of a culture made from the blood of the "original calf."

FIG. 27. *Trypanosoma evansi* from the calf found infected with surra.

PLATE VI.

FIG. 28. A group of the developmental forms of Koch; *Pirosoma bigeminum* on the second day of cultivation in bouillon; parasites lying between the red blood cells. Preparation from calf No. 7.

FIG. 29. Larger group of the same forms observed the same day as those illustrated in fig. 28. Parasites lying between red blood cells and leucocytes. Preparation from blood of calf No. 7.

FIG. 30. Ray form of Koch. Preparation from blood of calf No. 4.

FIGS. 31 and 32. Form with chromatin points described also by Koch. Preparation made from blood of calf No. 4 on the third day of cultivation.

FIG. 33. Large oval form with three chromatin masses and four chromatin tips, together with small developmental forms lying between the swollen erythrocytes. Preparation made from calf No. 4 on the third day of cultivation.

FIG. 34. Large oval form, lying isolated between swollen erythrocytes. Preparation from calf No. 4 on the third day of cultivation.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

PLATE I.

Fig. 1

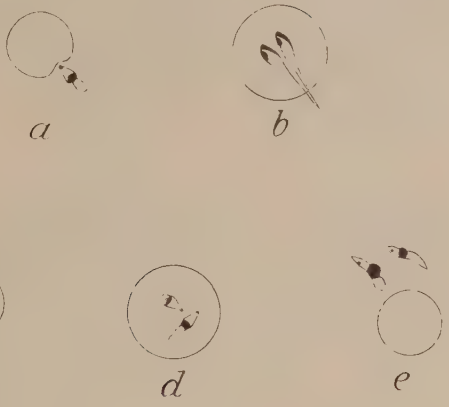

Fig. 2.

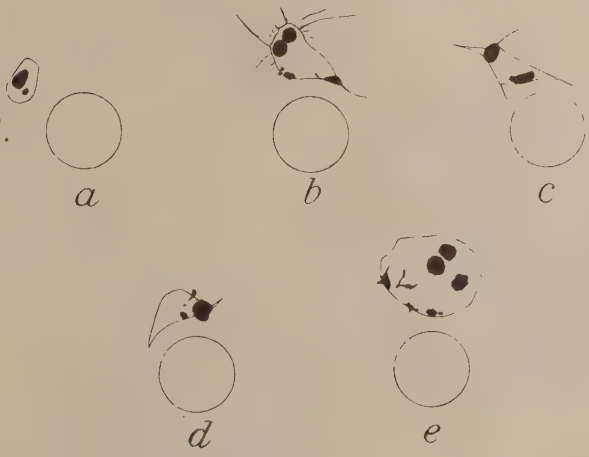

PLATE II.

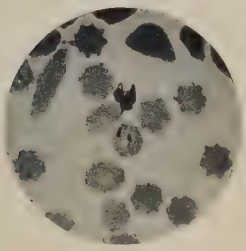

FIG. 1.

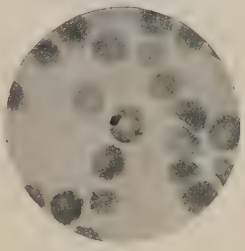

FIG. 2.

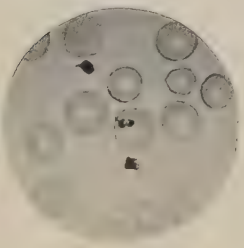

FIG. 3.

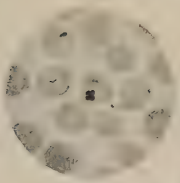

FIG. 4.

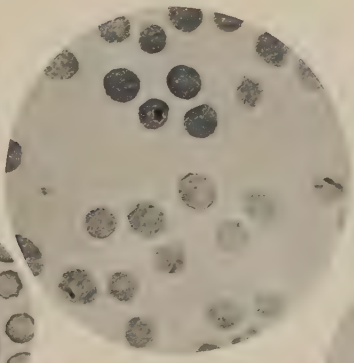

FIG. 5.

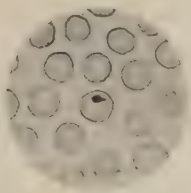

FIG. 6

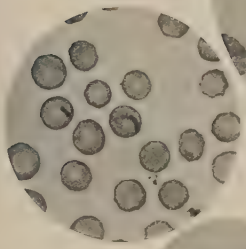

FIG. 7.

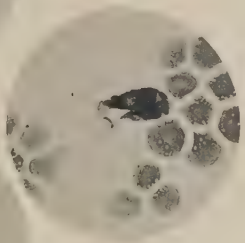

FIG. 10

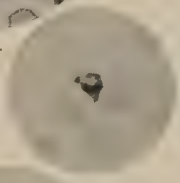

FIG. 8.

FIG. 9.

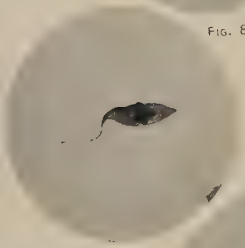

FIG. 11.

FIG. 13.

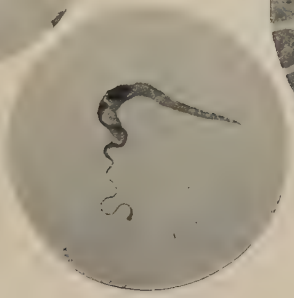

FIG. 12.

PLATE III.

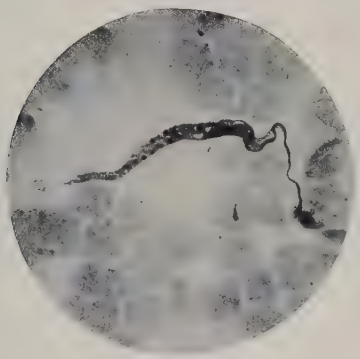

FIG. 14.

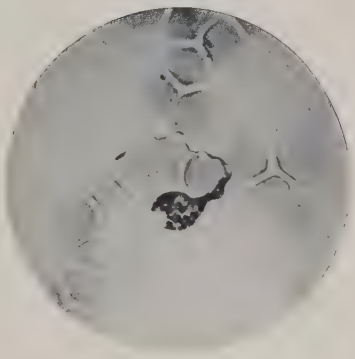

FIG. 15.

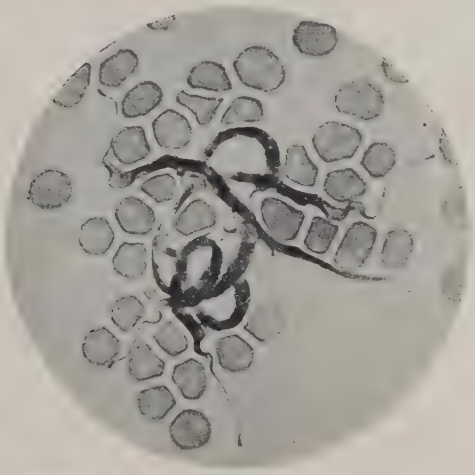

FIG. 18.

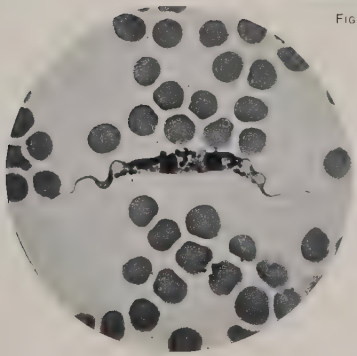

FIG. 16.

FIG. 17.

PLATE IV.

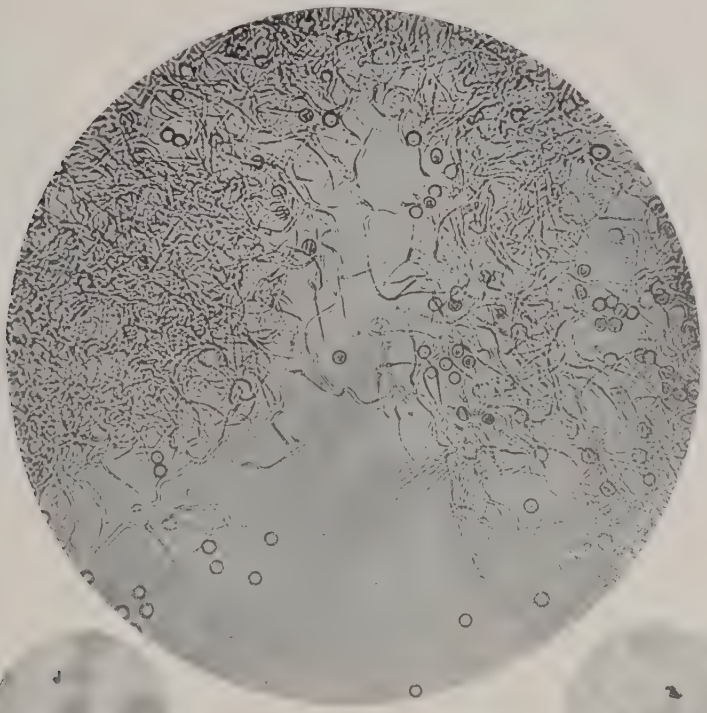

FIG. 19.

FIG. 20.

FIG. 21.

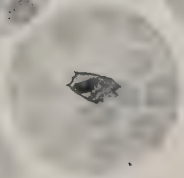

FIG. 24.

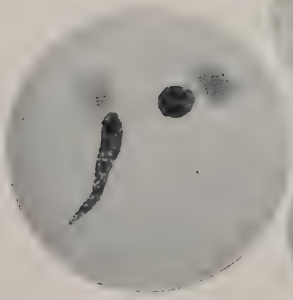

FIG. 26.

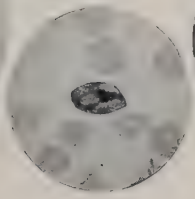

FIG. 25.

FIG. 22.

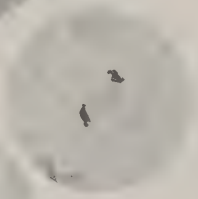

FIG. 23.

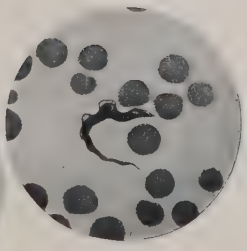

FIG. 27.

PLATE V.

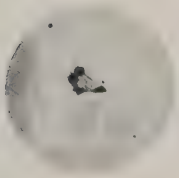

FIG. 30.

FIG. 28.

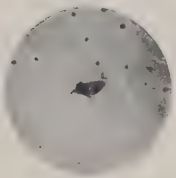

FIG. 31.

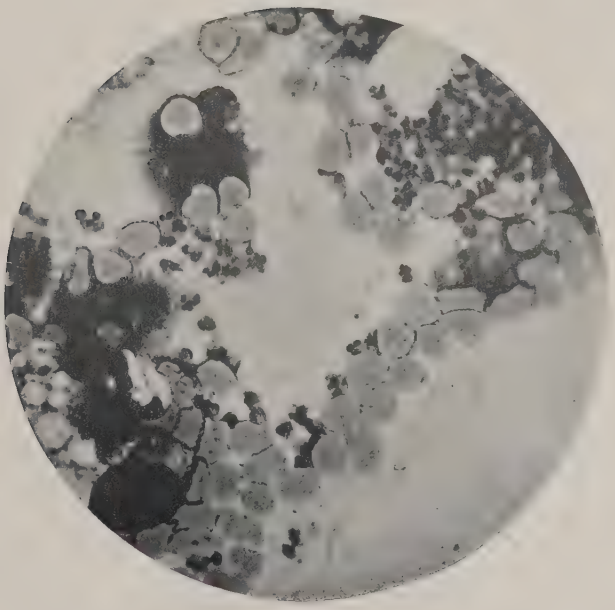

FIG. 29.

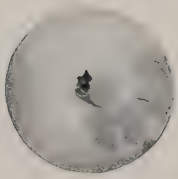

FIG. 32.

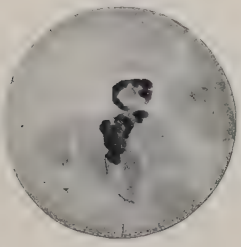

FIG. 33.

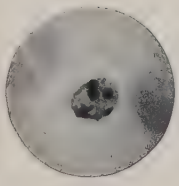

FIG. 34.

PLATE VI.

THE BACTERICIDAL SUBSTANCES IN FIBRIN.

By A. F. Coca.

(From the Biological Department of the Krebs Institut, Heidelberg (Professor von Dungern, Director), and the Biological Laboratory, Bureau of Science, Manila, P. I.)

The claim has been made, in recent years, that fibrin is a carrier of considerable quantities of bactericidal and hæmolytic substances and therefore is an important therapeutic means in the combating of infections, chiefly of a localized inflammatory nature.

In 1904 Ottolenghi [1] published the results of experiments with *Bac. anthracis* which seemed to show in the fibrin of certain animals—rabbit, ass, and horse—the presence of substances capable of reactivating an anti-anthrax serum. Such substances were lacking in the fibrin of the guinea pig, ox, and dog, the serum of which animals is, in this respect, likewise inert.

According to the later experiments of Sieber [2] nonspecific bactericidal substances can be obtained from fibrin by extraction with chloroform-thymol water during five to ten days.

A more recent contribution to this question has been made by S. Bergel,[3] who claims to have found both antibodies and complement in washed fibrin, and who was the first to make practical use of this element of the blood as a therapeutic agent. Proceeding upon the assumption that all the early tissue and vascular changes taking place in the process of inflammation have for their purpose the combating of the causative microörganisms and their toxic products, Bergel has endeavored to demonstrate in fibrin the presence of the two categories of bactericidal substances, namely, complement and immune body. Instead, however, of experimenting directly with pathogenic organisms, as his predecessors had done, he chose, as an analogous and more convenient system, red blood corpuscles and the specific hæmolysins and hæmagglutinins. A few experiments were also made with bacterial agglutinins.

The fibrin extract was prepared by Bergel in the following manner: blood drawn from a vein was whipped until all the fibrin had collected

[1] *Centralbl. f. Bakt., etc.* (1904), **37**, 584.
[2] *Ibid.* (1905), **38**, 571.
[3] *Deutsche med. Wcsnschr.* (1908), **34**, 369.

in a firm mass upon the stick used for whipping. This mass of fibrin was then cut into small bits and after having been washed well in physiological salt solution, was pressed between layers of filter paper, once more washed in the salt solution and suspended in a volume of that medium corresponding to the volume of the blood from which the fibrin had been derived. In a few cases the blood was not whipped, but centrifugalized as soon as possible after being drawn, and the fibrin obtained from the upper layer of coagulated plasma by simple expression of the serum. Instead of salt solution, glycerin was sometimes used for the extraction of the substance sought.

Extracts of fibrin obtained from guinea pig, rabbit, man, horse, and dog, were studied, and in these extracts were sought the normal and artificially produced hæmolysins (complement and immune-body) and the hæmagglutinins and bacterial agglutinins.

The results of the experiments were in every case positive, all the substances mentioned being found in one or another of the extracts examined.

On the basis of these experiments Bergel recommended the local injection of fibrin in the treatment of abscesses and "other surgical conditions" and reported that he had already obtained favorable results by this practice.

The latest study of this subject was made by Kindborg,[4] who approached it from a different standpoint from the other investigators.

The principal purpose of Kindborg's experiments was to demonstrate the power of fibrin to absorb bactericidal and hæmolytic substances of specific sera. In order to prove this he left the washed fibrin, which had been sterilized by moist heat, for various lengths of time in contact with bactericidal and hæmolytic serum, and determined in the sera thus treated the resulting diminution of these substances.

The experiments with bactericidal sera showed that their power to destroy the respective microörganisms was diminished by prolonged contact with fibrin, provided that the experiment was carried out at a temperature of 37° C.; at room temperature—averaging 10° C.—little or no effect was produced.

In interpreting this observation Kindborg considers only two possibilities, namely, the absorption either of antibody or of complement. He ignores the obvious possibility of substances being generated in the fibrin, particularly when the latter has been kept in contact with an active serum for many hours at body temperature—substances which are injurious to the labile complement. No experiments are reported in his article showing directly that it is not complement which is affected by contact with fibrin.

[4] *Centralbl. f. Bakt.* (1908), **48**, 335.

The experiments with a hæmolytic serum derived from a rabbit that had been immunized against bullock's blood corpuscles, showed, first, that the addition of sterilized bullock's fibrin to the serum deprived the latter of its specific dissolving power against bullock's blood and, secondly, that this effect was due to the absorption of the immune body.

In summing up the results of his investigations Kindborg assumes, without any discussion, that the action of the fibrin in both sets of experiments is identical. His conclusion, then, based upon the experiments with the hæmolytic immune serum, is that fibrin possesses a nonspecific power of absorbing the specific immune bodies. In all the experiments, however, upon which this conclusion rests the fibrin used was derived from the animal against whose blood corpuscles the immune body was directed, so that its ability to unite with that substance can by no means be regarded as nonspecific. It is not to be overlooked that fibrin holds in its meshes the stromata of both red blood corpuscles and blood platelets both of which possess a specific property of uniting with the corresponding antibodies.

Kindborg expresses surprise that Sieber should find bactericidal substances liberated by fibrin, whereas he himself has observed just the opposite action of this substance—a prevention of bacteriolysis. The explanation of this apparent contradiction is clear. Sieber subjected his fibrin to a prolonged autolysis and tested the products of this process directly upon the various microörganisms. Kindborg, on the other hand, treated his fibrin for a considerably shorter time by a similar process and exposed the much more sensitive complement to the injurious products.

Kindborg also questions Ottolenghi's conclusions, arguing that in his own experiments instead of a diminution of bactericidal property upon the addition of fibrin an increase of this power, according to Ottolenghi, must have resulted. To this argument Ottolenghi[5] replies that his conclusions are applicable only to the case of anti-anthrax serum and its special complement.

From this brief review of the literature we see that the experiments of Bergel are the only ones upon which the prospect for any practical use of fibrin as a therapeutic agent can be based.

In the course of some investigations carried out in the cancer institute in Heidelberg, I took occasion to repeat some of the experiments described by Bergel and by reason of the wide discrepancies between his results and my own and in view of the practical significance of the matter, I have thought it worth while to continue the study systematically. Part of this work was done in the biological laboratory, Bureau of Science, Manila.

In repeating Bergel's experiments the fibrin of rabbit and guinea pig has been used, both from normal and immunized animals, and the sub-

[5] *Ibid.* (1909), **49**, 615.

stances sought were complement, hæmolytic immune-body, and the hæmagglutinins, all of these being readily detected both qualitatively and quantitatively.

The method of preparing the fibrin extract was identical with that adopted by Bergel with the exception that sometimes the quantity of salt solution used for the extraction was less than the amount of blood yielding the fibrin.

Complement in fibrin.—The entire amount of fibrin obtained from 15 cubic centimeters of guinea pig's blood by whipping, after having been thoroughly washed in physiological salt solution and finely cut up with scissors, was suspended in 15 cubic centimeters of the salt solution and left for thirty hours at 15°; the serum from the same animal was kept at the same temperature.

The relative amount of hæmolytic complement in both serum and fibrin-extract was then determined in the usual manner:

Series of tubes A:

In all tubes 1 cubic centimeter 5 per cent sensitized bullock's blood corpuscles.
Diminishing quantities of fibrin-extract, 2.4 cubic centimeters, 1.6 cubic centimeters, .0.8 cubic centimeter, etc.

Series of tubes B:

In all tubes 1 cubic centimeter 5 per cent sensitized bullock's blood corpuscles.
Diminishing quantities of guinea pig's serum, 1/20 cubic centimeter, 1/40 cubic centimeter, 1/80 cubic centimeter, etc.

At the end of the usual period of observation no trace of hæmolysis was to be found in Series A, whereas in Series B 1/20, 1/40, and 1/80 cubic centimeter of serum had produced complete solution, 1/160 cubic centimeter moderate solution.

The amount of available complement in the fibrin from 2.4 cubic centimeters of guinea pig's blood is, therefore, less than that present in 1/160 cubic centimeter of the blood-serum of the same animal.

Hæmolytic immune-body in fibrin.—Rabbit No. 21 was given in the ear vein 5 cubic centimeters of washed bullock's blood corpuscles and two and one-half weeks later a similar injection of 10 cubic centimeters. Eight days after the second injection the animal was killed and the fibrin from 50 cubic centimeters of its blood used for the following experiment. The fibrin-extract was prepared as before, only 10 cubic centimeters of salt solution being used for the extraction.

Series of tubes A:

In each tube 1 cubic centimeter 5 per cent bullock's blood corpuscles.
Diminishing quantities of fibrin extract, 4/10, 2/10 cubic centimeter, etc., to 1/160 cubic centimeter.

Series of tubes B:

In each tube 1 cubic centimeter 5 per cent bullock's blood corpuscles.
Diminishing quantities of serum 1/160, 1/320 cubic centimeter, etc., to 1/1280 cubic centimeter.

After one and one-half hours at room temperature no hæmolysis having occurred in any of the tubes, all of them were centrifugalized and the fluid poured off. To each of the tubes were then added 1/10 cubic centimeter of normal rabbit serum and 1 cubic centimeter of physiological salt solution. After two hours at 37° C. and twenty hours at room temperature, in series A complete solution had taken place in the tube containing 4/10 cubic centimeter of fibrin extract; 2/10 cubic centimeter had produced strong solution, 1/10 cubic centimeter none. In series B complete solution had been produced by 1/160 and 1/320 cubic centimeter of serum, almost complete solution by 1/640 cubic centimeter, and very strong solution by 1/1280 cubic centimeter of serum.

The entire amount of fibrin, therefore, in 50 cubic centimeters of the blood of rabbit No. 21 had yielded a quantity of hæmolytic immune-body equivalent to that possessed by 1/25 cubic centimeter of the same animal's serum.

Hæmagglutinins in fibrin.—Rabbit No. 11 was given the washed corpuscles of 10 cubic centimeters of chicken's blood into the ear-vein and ten days later was bled from the jugular vein; for the following experiment the fibrin from 30 cubic centimeters of the blood was obtained by whipping. For the extraction, which lasted twenty-four hours, 10 cubic centimeters of salt solution were used.

It was found that whereas 1/20 cubic centimter of the inactivated serum produced complete agglutination of 1 cubic centimeter of a 5 per cent suspension of chicken's blood corpuscles, it required 1.6 cubic centimeters of the extract to agglutinate completely 1 cubic centimeter of the same suspension, while 0.8 cubic centimeter agglutinated slightly and 0.4 cubic centimeter not at all.

Therefore the amount of agglutinins available in the fibrin from 30 cubic centimeters of this blood equaled that found in about 1/3 cubic centimeter of the same animal's blood-serum.

These experiments and others of the same nature were often repeated and always with the same result, namely, *that there is an incomparably greater quantity of the substances under consideration in the serum than in the fibrin of the same specimen of blood.* Consequently, the logical conclusion of this study is, that if the benefit derived from fibrin injections is dependent upon the classes of substances under investigation, it must be considerably more advantageous to use the corresponding sera for the purpose of treatment. However, when we remember how unsatisfactory, as yet, the treatment has been with antistreptococcic and antistaphylococcic sera, and it is the microörganisms with which these sera are prepared which are chiefly responsible for the local inflammations, it becomes apparent that if the injected fibrin does prove to be efficacious in the cases mentioned, it will not be because it carries bactericidal substances.

THE DEVELOPMENT OF THE MIRACIDIUM OF PARAGONIMUS UNDER VARIOUS PHYSICAL CONDITIONS.[1]

By PHILIP E. GARRISON[2] and RICARDO LEYNES.[3]
(*From the Biological Laboratory, Bureau of Science.*)

INTRODUCTION.

The present paper presents observations made during the past two years upon the development of the ova of *Paragonimus* under various physical conditions. It is in part based upon the repetition on a more extensive scale of the experiments and observations of Manson (1882), Kellicott (1894), Kerbert (1881), Otani (1887), Miura (1889), and Nakahama (1883), and in a large part upon experimental work which, so far as we are aware, has not been previously done.

The work was undertaken with three practical purposes in mind, namely, to add to our knowledge concerning, first, the prophylactic treatment of paragonimiasis, especially as regards the care of infected patients; secondly, the possibility of introducing the lung-fluke into colder climates; and thirdly, the life-cycle of the parasite, notably, its probable intermediate host.

With these purposes in view experiments have been made, first, to determine the most favorable conditions for the development of the ova and the time required for such development; secondly, their resistance to varying degrees of temperature and light, to desiccation, and to salt solution of various strengths.

MATERIAL.

Sputum and fæces containing *Paragonimus* eggs have been used from about fifteen different patients, one, a patient at the Civil Hospital, the others in the hospital at Bilibid Prison. By far the greater number of experiments have been made with the sputum of a Bilibid prisoner, number 3680–P, who for over a year past (when he came under observation) has expectorated daily from 10 to 30 cubic centimeters of blood-tinged, mucoid sputum, heavily loaded with *Paragonimus* ova.

[1] Read at the Sixth Annual meeting of the Philippine Islands Medical Association, February 13, 1909.

[2] Assistant surgeon, United States Navy; detailed medical zoölogist to the Biological Laboratory, Bureau of Science.

[3] Student demonstrator in Medical Zoölogy, Philippine Medical School.

NORMAL DEVELOPMENT.

The ova of *Paragonimus* were first developed to the free miracidial stage by Manson in 1880, who after shaking the sputum with water and renewing the water daily for about a week found the motile miracidia developed in from four to six weeks after the sputum was expectorated. The favorable results of such a method were entirely in harmony with what was known of the development of the ova of other digenetic trematodes and in our hands the method has never failed throughout a series of several hundred such cultivations of the ova, provided two important requirements were complied with, namely, that the washing of the ova be thorough and that it be not too long delayed after the sputum is expectorated.

Sedimentation.—The method we have used for clearing the ova from the sputum or fæces is as follows:

The specimen (sputum or fæces) is placed in a tall museum jar, holding about 2,500 cubic centimeters and about 10 centimeters in diameter. Such a jar while holding a good volume of water confines the sediment to a comparatively limited area. Tap water is run into the jar as violently as possible in order to break up the mucoid or solid parts of the specimen and the jar allowed to stand until the ova have sunk to the bottom, which usually happens in from one to four or five hours, depending upon the specific gravity of the solution.

If the specimen be sputum, after standing for an hour or two, the water is decanted off to as close to the sediment as possible and the jar refilled and allowed to stand until the following day, when the water is again changed. If, after sedimentation has taken place, the water is perfectly clear it is poured off and the sediment transferred to a bottle of half a liter capacity or less and not again disturbed, unless the water it contains becomes clouded or covered with a scum by the excessive growth of bacteria or other organisms.

Fæces are more difficult to wash satisfactorily. In the case of stools with a large amount of soluble matter, we have frequently used 10 liters or more of water in order to get the first solution sufficiently light to allow the ova to settle and it was found to be necessary to change the water repeatedly until the fæcal character of the stool apparently disappears. When once thoroughly washed, the ova from fæces develop as well apparently as those from sputum, but we have frequently failed to get them sufficiently and quickly clean and both for this reason and also because of their bulk and of the larger amount of sediment remaining fæcal specimens have been little used in our experimental work.

Conditions of temperature and light.—Cultures of the ova, including those used as controls for experiments, regularly have been kept uncovered, out of direct sunlight, upon a laboratory shelf, where the temperature varies from 25° to 34° C.

Since thoroughly washed ova kept under these conditions developed satisfactorily and as under no variation from these conditions was development more constant, more rapid, or apparently more healthy, we have used the maximum development so obtained as the standard for comparison and considered it, at least for the laboratory, as normal.

Time required.—Manson found developed miracidia in from four

to six weeks, at a temperature of from 26°.7 to 34°.4 C. Nakahama (1883) reported that the ciliated miracidium developed in twenty-eight days, at a temperature of 30° C.

In our own cultivations we have endeavored to record the length of time from the day the sputum was expectorated to (1) the day of the first motile miracidia, (2) the day of the first free swimming miracidia, and (3) the day the last motile miracidia were found in the culture.

Referring to the observation of Kerbert (1881) of the presence of ova in the uterus of the worm developed to the gastrula stage, and to that of Manson (1882) of ova segmented several times in the sputum, we may say in this connection that in freshly expectorated sputum we have never been able to detect segmentation of the germ-cell.

We have repeatedly been able to grow the motile miracidium in fifteen days from the time the sputum was expectorated, but in order to do this, it was essential that the ova be thoroughly sedimented on the day the specimen was obtained and that the water be promptly changed whenever it became at all clouded. Any delay in the first washing or failure to keep the water clean resulted in a longer period of development, though not necessarily in degeneration.

All cultivations, although the most favorable conditions were complied with, did not develop the motile miracidium in so short a time, some requiring from twenty to twenty-five days.

Such variations did not appear to correspond to such slight differences of temperature as occurred in the laboratory at different times of the year. Motile miracidia have developed in fifteen days, while the thermometer ranged from 25° to 28° C.; at other times, with a temperature of from 29° to 31° the development was slower.

Therefore it is apparent that a difference in temperature does not explain the more rapid development obtained in our cultivations than it did in those of Manson and of Nakahama. From our experience it would appear that a more important factor than temperature within the limits indicated is the prompt and thorough washing of the ova and the cleanliness of the water in which they grow.

We have never found free-swimming miracidia in our cultures in less than twenty-five days from the time the sputum was expectorated, but in from twenty-five to thirty-five days they have frequently been noted. Therefore it is evident that the miracidia require, after they first acquire motility, a considerable period for further development before they are capable of leaving the shell.

A more striking observation is the length of time motile miracidia may remain in the shell before hatching. While as a rule practically all the shells in a culture thirty days old, contained actively motile miracidia, active, unhatched organisms would persist in the same culture for one hundred and fifty days, and in one case we found shells containing motile

miracidia in a culture one hundred and sixty days (twenty-three weeks) old. In cultures kept a longer time than this, we were never able to find anything but empty shells or degenerated ova.

Therefore it would appear that while the ova develop comparatively uniformly until the miracidia are, to all appearances, fully mature, the escape of the miracidia from their shells is, for a given number of ova, a matter of considerable variation and that the hatching of the ova thrown out in a single expectoration may be distributed throughout a considerable period of time—according to our experience, seventeen to eighteen weeks. This observation appears to explain the fact that we have never been able to find a great number of free-swimming miracidia in our cultures at any one time, even though the sediment was very heavily loaded with ova and it is possible that the gradual hatching of the ova may prove to be not without significance when the complete life-cycle of the parasite is known.

Having determined the laboratory conditions under which development of the ova of the lung-fluke was most favorable and observed the time and manner of such development, the remaining experiments, with which we are here concerned, were performed with the idea of ascertaining the variations from these conditions under which development would still take place.

DEVELOPMENT UNDER VARIOUS CONDITIONS OF TEMPERATURE.

Cultures placed in the incubator at body temperature (37° to 38° C.) not only showed no development, but rapidly degenerated. Subjected to such a temperature for ten days, the cellular content of the ova appeared broken down into an amorphous mass of granules and, if removed from the incubator, gave no evidence of development though kept beside a control culture for several weeks.

Cultures kept at room temperature until the miracidia were developed and then placed in the incubator at 37° gave similar results. Within an hour the organisms became quiescent, although those on a control slide were still actively motile. Within a few days they became broken down into a mass of granular débris.

The results of these two experiments would seem to be at least presumptive arguments against the reported observation of partly developed ova in the tissues of the final host of the parasite (see Kellicott, 1894) and also against the possibility more recently suggested (see Manson, 1908) that the miracidia of *Paragonimus* might be the infecting stage for man.

Cultures kept in cold storage at from 11° to 15° C., 10° to 12° C., and from 9° to 10° C. gave no signs of development after ten weeks, but likewise no degeneration, and when removed from the cold storage to room temperature, never failed to develop motile miracidia in about

the same length of time after leaving the cold chest as was required for its original control at room temperature.

It would appear, therefore, that while temperatures above 15° C. are required for the development of *Paragonimus* ova, temperatures as low as 10° do not destroy or, apparently, even impair their vitality.

Ova from the fresh sputum, when frozen solid and immediately thawed, apparently developed as well as did their controls. Likewise, ova frozen solid for five or six minutes seemed uninjured, and, although at times the proportion of undeveloped ova seemed somewhat larger than in the unfrozen controls, the difference certainly was very slight and the greater number of the eggs developed apparently as promptly and as normally as those in the controls. Ova frozen longer than five to six minutes, however, began to show injury, in that development was apt to be delayed and an unmistakably higher proportion failed to develop. Nevertheless, we have had cultures which were frozen for ten minutes develop apparently as well as the control ones, though, perhaps a few days later; other cultures, frozen approximately the same length of time, have shown a high percentage of degenerate ova. Ova frozen fifteen, twenty, and twenty-five minutes showed with fair consistency an increasing proportion of ova which failed to develop, and those frozen thirty minutes revealed only a few motile miracidia and these required about forty days for development. No ova after being frozen solid for over one-half hour gave any signs of development.

The results obtained from freezing the developed miracidia were very similar. The actively motile organisms in their shells could be frozen solid for five minutes, with impunity, resuming their activity when thawed. If frozen for ten minutes, however, only about one-half remained motile, and if for fifteen to twenty minutes, perhaps one-third, or less. Motile miracidia frozen twenty-five minutes or longer have consistently failed to retain their motility when thawed, and have invariably died and degenerated.

If the freezing be repeated after once thawing, the effect is more pronounced. A culture of motile miracidia, frozen, immediately thawed, refrozen for five minutes and again thawed, showed but a few motile organisms remaining, and these appeared to have died by the next day, when no motile miracidia could be found.

DEVELOPMENT UNDER VARIOUS CONDITIONS OF LIGHT.

Ova exposed to direct sunlight rapidly degenerated, and ova from which light was absolutely excluded developed practically step by step with the controls which were exposed to the reflected light of the laboratory room.

Referring to the observation of Looss (1890), that the miracidia of the conical amphistome (*Amphistoma cervi*) of cattle and sheep escape from

the shell only when exposed to light, we would say that we have repeatedly found from three to five free-swimming miracidia in one cover-glass preparation from a culture of *Paragonimus* ova which had been kept in a sealed stone jar and which had been exposed to light only for the fraction of a minute necessary to place a drop of the sediment under the microscope.

Therefore it would appear not only that direct sunlight is fatal to the life of the ovum, but that the presence of any light is not necessary to its development, at least to the free-swimming, miracidial stage.

EFFECT OF SALT SOLUTIONS.

The ability of the ova of the lung-fluke to develop in salt or brackish water would not be without its important practical bearings. In sea water, taken from Manila Bay, the eggs invariably and rapidly degenerate. Such water contains about 3 per cent of sodium-chloride. In solutions of commercial salt, containing 1.5 per cent or more of the salt, no development took place. In 1 per cent solutions, from one-third to one-half the ova developed motile miracidia, the others degenerating. In 0.5 per cent solutions the development was nearly as good as in the tap-water controls, though degenerated eggs appeared to be more frequently encountered. In neither the 0.5 or 1 per cent solutions were free-swimming miracidia ever seen.

DESICCATION.

Aside from the actual demonstration of the intermediate host and the mode of infection of *Paragonimus*, there is perhaps no question more important in paragonimiasis than the possibility of the dissemination of the ova in dried sputum or dust.

As remarked by Stiles, the long retention of the ova in the moist sputum, corresponding to the experimental conditions of Manson, would seldom occur in nature. The natural fate of the ova expectorated in the sputum of the ambulatory paragonimiasis patient (and most such patients are ambulatory) would be either for them to be washed into a body of water or, having been washed free from, or while still retained in the sputum, to become dried on the surface of the ground. It was with a view of determining the probable fate of these ova which become dried that we made a study of experimentally dried ova.

All ova which were allowed to become dry, even for a few minutes, by evaporation at room temperature, failed to develop thereafter.

Such a result was surprising in view of the great power of resistance to desiccation shown by the ova of certain other parasites, but repetitions of the test consistently gave the same result.

In our first tests, the cultures were allowed to remain dry for from one to forty days before the water was renewed. All of the ova degenerated, without showing signs of development.

Cultures were then left dry for from one to several hours, with the same results.

Finally, cultures in petri dishes were carefully watched until the last water had evaporated and the sediment was left dry in the bottom. After a short interval, which approximated and certainly did not exceed ten minutes, fresh water was added. When examined under the microscope, the shells were shrunken and the cellular contents broken down and though the cultures were kept under observation for several weeks after the control-cultures had developed motile miracidia, no development occurred in those previously dried.

It would seem safe to conclude, therefore, that desiccation, even for a few moments, is fatal to the life of *Paragonimus* eggs and that the ova can not be disseminated otherwise than in water.

The theory which has been broached that infection of man might occur by means of ova blown about in the air, even though there were no other objections to it, would appear to be absolutely excluded by the failure of the ova to withstand drying.

REFERENCES.

For complete bibliographical references to articles quoted see Stiles and Hassall, Index Catalogue of Medical and Veterinary Zoölogy, Bulletins of Bureau of Animal Industry, Washington, D. C.

THE INTESTINAL WORMS OF 385 FILIPINO WOMEN AND CHILDREN IN MANILA.[1]

By Philip E. Garrison[2] and Rosendo Llamas.[3]

(*From the Biological Laboratory, Bureau of Science.*)

Last year a paper was presented before this association giving the results of the examination of over 4,000 adult, male, Filipinos for the prevalence of animal parasites.[4]

The differences which have been reported by numerous authors between the frequency of infection with the various species of parasitic worms in males and in females and in adults and in children were used at that time to make a general forecast of what frequency of infection might be expected in Filipino women and children. However, it remained to definitely inform ourselves concerning the parasites of women and children by actual examination. The results here reported are the first step in that endeavor and are based upon the examination of 385 women and children in Manila. The examinations were made at Bilibid Prison, Hospicio de San Jose, St. Paul's Hospital, and the School for Deaf and Dumb Children of the Bureau of Education, and to these institutions we would acknowledge our indebtedness for the facilities provided.

Of the total 385 persons examined, 342 or 89 per cent were infected with intestinal worms as against 84 per cent of the male prisoners at Bilibid. The total number of infections found in the 385 women and children was 533, or 138.7 infections to each 100 persons examined, against 142 infections with intestinal worms in each 100 men.

Of the 385 persons examined, 227 were women—and of these alone

[1] Read at the Sixth Annual Meeting of the Philippine Islands Medical Association, Manila, February 13, 1909.

[2] Asistant surgeon, United States Navy; detailed medical zoölogist to the Biological Laboratory, Bureau of Science.

[3] Student assistant in medical zoölogy, Philippine Medical School.

[4] Among the 385 women and children examined, amœba were found 37 times (10.8 per cent) and other intestinal protozoa 24 times (7.0 per cent). These figures are excluded from our statistics since the examinations were frequently made under conditions unfavorable for diagnosing these infections, most often owing to the lapse of time after the specimen was passed before the examination could be made.

192 were infected, or 85 per cent—while of the remaining 158 children, 150, or 95 per cent, were infected. The women gave 291 infections with intestinal worms or 128 infections per 100 examined; while the children gave 242 infections or 153 per 100.

The infections with each parasite found were as follows:

	Infections.	Per cent.
Trichuris	300	87.60
Ascaris	182	53.22
Hookworms	46	13.45
Strongyloides	2	.6
Oxyuris	2	.6
Tænia	1	.3

A comparison of the frequency of these parasites in men from all over the Islands and women and children in Manila is shown in the following table:

Animal parasites.	Men.	Women.	Children.
	Per cent.	*Per cent.*	*Per cent.*
Trichuris	59.00	84.00	92.00
Ascaris	26.00	51.00	56.00
Hookworms	52.00	15.00	11.00
Strongyloides	3.00	0	0
Oxyuris	0.8	0	1.33
Tænia	0.7	0	.66
Hymenolepis	0.1	0	0

A higher rate of infection with *Ascaris* and *Trichuris* among women and children than among men was anticipated from the relative frequency of these worms in the two sexes and among different ages in other localities. The frequency of hookworms is strikingly lower than was found among the Bilibid prisoners.

The infections with the other parasites are too few for purposes of comparison. In view of the fact that the greater frequency of *Hymenolepis* among children than among adults has been recorded by several authors, it is worthy of note that no infections with the dwarf tapeworm were encountered among the 158 children examined, although it was found in about 1 per cent of the adult males examined at Bilibid Prison.

RELATION OF THE INDIAN FORM OF RELAPSING FEVER TO AFRICAN TICK FEVER.[1]

By Richard P. Strong.
(*From the Biological Laboratory, Bureau of Science.*)

In 1904 Manson,[2] after examining the blood of a patient from Gibraltar suffering from her eighth paroxysm of relapsing fever, suggested, on the ground of the unusually large number of relapses and the locality in which the infection was acquired, that there might be several forms of this type of disease due to different species or varieties of spirochætæ. Ross and Milne [3] in the same year stated that it was possible there might be more than one form of tick fever, and Sambon [4] suggested that a spirillum which was so widely distributed and fostered by different invertebrate hosts in different countries might be represented by a number of more or less distinct varieties or species. In 1906 Novy and Knapp [5] studied a case of relapsing fever in the United States, and on account of morphological characteristics which they were able to detect in several stained specimens of the spirochæta from their own case, and in those of African spirochætæ obtained by them from the Liverpool School of Tropical Medicine, concluded that relapsing fever and tick fever are distinct. They also based this claim upon the published experiments of Dutton and Todd [6] and particularly upon those of Breinl and Kinghorn,[7] who found that the spirochæta of the tick variety was frequently fatal to rats and mice and that in rats from three to four relapses occurred before death. Novy and Knapp [8] found that in the case of the spirochæta, which they regarded as *Spirillum obermeieri,* the infection in rats was of shorter duration and that no relapses occurred. They also believed that

[1] Read at the Sixth Annual Meeting of the Philippine Islands Medical Association, February 13, 1909.

[2] *Brit. Med. Journ.* (1904), **1**, 538.

[3] *Brit. Med. Journ.* (1904), **2**, 1453.

[4] *Brit. Med. Journ.* (1905), **2**, 1266.

[5] *Journ. Am. Med. Ass.* (1906), **46**, 116; *Journ. Infect. Dis.* (1906), **3**, 291.

[6] *Brit. Med. Journ.* (1905), **2**, 1259; *Mem. Liv. School of Trop. Med.* (1905), **17**, 1.

[7] *Lancet* (1906), **1**, 668; *Mem. Liv. School Trop. Med.*, **20**, 61.

[8] *Loc. cit.*

the diffuse flagella of the organism of tick fever as pictured by Zettnow [9] served as an additional "clinching" proof and effectually differentiated it from *Sp. obermeieri* which had, according to their observations, but a single terminal flagellum. Uhlenhuth and Hændel [10] later claimed that Novy was not working with *Spirochæta obermeieri,* but with another species, an American variety. Fränkel [11] claimed that numerous flagella were present on Novy's organism. Breinl and Kinghorn [12] found that a monkey and several rats immunized against the American spirochæta (supposed to be identical with *Sp. obermeieri*) remained susceptible to the African species. They were also able to infect a horse, dogs, rabbits, guinea pigs and other animals with the tick-fever parasite. They therefore concluded that the two varieties, American and African relapsing fever, are distinct. In 1896 and 1897 Gabritschewsky [13] and Loeventhal [14] suggested and employed the serum for diagnostic purposes in cases of relapsing fever, observing its bactericidal and agglutinative reaction under the microscope. Later Karlinski,[15] Routkewitsch,[16] Mielkich [17] and particularly Hodlmoser [18] also employed this method for diagnostic purposes, though not always with satisfactory results. In 1906 Novy and Knapp proposed to differentiate the different species of spirochætæ by serum reactions, specific agglutinins and bacteriolysins, as well as by animal inoculations. Uhlenhuth and Haendel [10] and Fränkel,[19] during the past year, and very recently Manteufel,[20] by means of animal inoculations as well as by agglutinative and bacteriolytic reactions, have found that different results are obtained with the European, African and American spirochætæ, and they regard them as three distinct species.

I have emphasized elsewhere [21] the difficulties encountered in performing agglutinative and bacteriolytic tests with the spirochætæ of this group and the frequency with which pseudo-reactions occur. By far the most accurate method of differentiation of these species of spirochætæ is by the inoculation, with the strain of the spirochæta to be

[9] *Ztschr. f. Hyg. u. Infectionskrankh.* (1906), **52,** 539.

[10] *Arb. a. d. k. Gsndtsamte* (1907), **26,** Heft I, 1.

[11] *Hyg. Rundschau* (1907) **17,** 263.

[12] *Lancet* (1906), **1,** 1690; *Mem. Liv. School Trop. Med.* (1906), **20,** 61, 69, and **21,** 1.

[13] *Ann. Inst. Past.* (1896), **10,** 630.

[14] *Deutsche med. Wchnsch.* (1897), **23,** 560.

[15] *Wien. klin. Wchnsch.* (1903), **16,** 447; *Centrbl. f. Bakt.,* etc. (1902), abt. 1, **31,** 566.

[16] *Baumg. Jahresb.* (1898), **14,** 613.

[17] *Baumg. Jahresb.* (1900), **16,** 434.

[18] *Wien. med. Wchnsch.* (1904), **54,** 2310; *Zeitschr. f. Heilh. Abt. Interne Med.* 1905 new series **6,** 506.

[19] *Berl. klin. Wchnsch.* (1907), **44,** 681.

[20] *Arb. a. d. k. Gsndhtsamte* (1907), **27,** Hefte II., 327.

[21] *This Journ. Sec. B.* (1908), **3,** 231.

tested, of an animal which has already been rendered thoroughly immune to the strain of spirochæta supposedly different. Schellack [22] has recently described the morphological differences in the European, American and African spirochætæ of the recurring fevers but Leishman [23] could not make any distinction between them from a morphological standpoint. Kolle and Schatiloff [24] have reported upon the use of the complement fixation reaction with human immune serum, from cases convalescent from relapsing fever, for the purpose of the differentiation of the different species.

The question then suggested itself whether the form of Indian relapsing fever, known as Bombay spirillum fever constituted another disease, or one which was caused by one of those species of spirochætæ already recognized and described. This fever has been known to be very common in India since 1852 and was carefully described by Van Dyke Carter in 1877.[25] The important descriptions which have been previously published upon the relation which this fever bears to the European relapsing fever are those of Novy and Knapp [26] and of Mackie [27] of Bombay. Novy and Knapp, after a study of several blood smears sent them by Patton from Bombay, concluded from several very minor differences in morphology, such as slight variations in thickness (the American spirillum appearing thicker), and a tendency of the Indian spirillum to form loops, particularly in agglutination, and to show multiple transverse division, that perhaps the Indian strain constituted a new species, different from the American one. Novy and Knapp admitted, however, that there might be some question about the Indian variety of spirochæta representing a distinct species and in the absence of fresh material they were unable to settle the question. They stated that several division zones like those of the Bombay organism, and like those present in *Spirochæta Duttonni*, are not found in the American species of spirochæta. However, Oppenheimer [28] found these same phenomena in the American species. She concludes that—

"1. The New York *Spirochæta Obermeieri* can not yet, as has been attempted, be separated from the African spirochæta, upon the following grounds: (1) The length of its stay in the peripheral blood of the rat, (2) the number of relapses in the rat, (3) the lack of figure-8 and circular forms, (4) the absence of several transverse breaks; for the length of stay in the peripheral blood probably varies with the method of passage, relapses are an uncertain quantity since it is perhaps not positively established that they occur at all; figure-8 forms and circles and finally several division zones exist in the New York spirillum as well as in *Sp. Duttoni* and in the spirillum of Bombay."

[22] *Arb. a. d. k. Gsndtsamte* (1907), **27** Heft II., 364.
[23] *Lancet* (1907), **1**, 806.
[24] *Deutsche med. Wchnsch.* (1908), **34**, 1176.
[25] *Med. Chir. Trans.* (1877), **41**, 274.
[26] *Loc. cit.*
[27] *Lancet* (1907), **2**, 832.
[28] *Collected Studies Research Lab., Dept. Health*, N. Y. (1906), **2**, 146.

The published description of Mackie, who had plenty of material at his disposal for study, also does not lend support to the morphological differences which Novy described for the Indian spirochæta. Mackie found the American spirochæta thinner than the Asiatic one. Novy, moreover, conjectured that the Indian species possessed diffuse flagella, while Mackie describes the appearance of only a single flagellum in the Indian species, which, however, he regards as a collapsed sheath rather than a true flagellum. Mackie performed some agglutinating experiments with an agglutinating serum prepared against the American spirochæta and sent him by Novy. However, the serum failed to agglutinate the Bombay spirochæta. Whether this was due to the fact that the serum had lost its agglutinating properties after leaving Novy's laboratory, or that a more specific complement might have been necessary than that supplied by the Bombay rat, or to the fact that the Bombay spirochæta was of a different variety, Mackie could not state. I have already called attention to the uncertainty in obtaining the agglutination test with these spirochætæ. In a later article [29] Mackie has summarized the evidence he was able to collect from the literature and made a comparison of all the strains.

I therefore determined to try to throw further light upon the question of the nature of Bombay spirillum fever. The various strains of spirochætæ—African, American and European—were collected during my travels in the different countries. I desire to thank Professors Prowazek of Hamburg, Flexner of New York, and Dryer of Egypt for supplying me from their respective laboratories with strains of these organisms. As most of you are aware, these spirochætæ can only be kept alive successfully in the animal body and can not be cultivated in the test tube on artificial media.[30] White mice are the only animals satisfactorily susceptible to all of the species. It was therefore necessary, in order to carry out this work, to travel with a plentiful supply of these animals as well as with a supply of white rats. Moreover, in order to be sure not to lose any of the strains, it was found necessary to inoculate with each strain a fresh mouse with the infected blood of another animal every third or fourth day. There were some difficulties encountered in carrying out these successive inoculations while traveling from continent to continent during a period of four months. In passing, it may be of interest to remark that in Egypt I was able to find and identify several cases of relapsing fever which were caused by the European variety of spirochætæ, as well as a case of African tick fever caused by the *Spirochæta Duttoni*.

[29] *N. Y. Med. Jour.* (1908), **88**, 337.

[30] The collodion sac method of cultivation in the abdominal cavity of an animal, as descibed by Levaditi and subsequently by Novy and Knapp, is too uncertain to be always relied upon.

When I finally reached Bombay with my animals, I was unable to find any cases of relapsing fever there. Only just before my departure from that city several cases occurred in the Hospital for Infectious Diseases, presided over by Doctor Choksy. However, these cases were evidently in a stage of relapse and their blood was of no value for experimental purposes. As my supply of white mice was by this time exhausted, my experiments in relation to the Indian form of relapsing fever would have failed but for the timely assistance of Captain Mackie of the Indian Medical Service of Bombay. Fortunately I had secured in Egypt through the kindness of Dr. Keatinge, director of the Cairo School of Medicine, some white rats which, during the voyage to India, I had immunized against the different spirochætæ. At my request, Captain Mackie kindly consented, when a fresh case of the Indian disease should occur, to attempt to infect with the Indian spirillum my rats which had been immunized separately against the African (Koch and Dutton strains), European and American strains of spirochætæ. Each animal had been highly immunized with one of these strains by repeated injections of blood containing the spirochæta in question and the animals were no longer capable of being infected with the respective organisms. Captain Mackie was unable to obtain suitable blood containing the Indian species of spirochæta until forty-eight days after my last injection of the rats with the spirochætæ, at this time, however, the attempt was made to infect them. As immunity in such animals has been shown to persist for many months by Novy, Manteufel and myself, they were evidently still immune to the strain with which they had been previously inoculated at the time the attempt to reinfect them was carried out by Doctor Mackie. The rats were all inoculated with about 0.4 cubic centimeter of pure blood in citrate solution. The blood at the time of the inoculation contained numerous actively motile spirochætæ. All of the injections were made intraperitoneally. Control, normal rats were also inoculated at the same time. Twenty-four hours after the injections were made a microscopic examination of the blood of the animals showed that no spirochætæ were present in those immunized with the American and with the European strains, but that spirochætæ were present in the blood of all those animals immunized against the African strains as well as in the blood of the control normal animals. Forty-eight hours after infection the spirochætæ were still present in the blood of one of the animals immunized against the African species, and, as might be expected, in one of the control, normal animals; in all of the others the spirochætæ had disappeared. An examination seventy-two hours after infection showed the blood of all the animals negative for parasites and the organisms did not appear or reappear again in any, as was evidenced by repeated careful examinations. I take this opportunity publicly to thank Captain Mackie

for kindly carrying out these inoculations. The details of the experiments are as follows:

EXPERIMENTS ON IMMUNIZED RATS REINOCULATED WITH "SPIRILLUM CARTERI" OF INDIAN RELAPSING FEVER.

Rat No. 1, immunized against Koch's African spirochæta.
Rat No. 2, immunized against American spirochæta.
Rat No. 3, immunized against European spirochæta.
Rat No. 4, immunized against Dutton's African spirochæta.
Mouse No. 5, immunized against Koch's African spirochæta.
Rat No. 6, nonimmune (*Mus Rattus*, Bombay).
Rat No. 7, nonimmune (*Mus Rattus*, Bombay).
Monkey, nonimmune (*Macacus Sinicus*).

The material with which the animals were inoculated was obtained from a monkey (*Macacus Sinicus*), No. 85, whose blood contained many active spirilla and which was at the height or just before the height of the attack (thirty-six hours before crisis). An equal quantity (about 0.4 cubic centimeter of pure blood) of this citrated blood was injected into animals Nos. 1, 2, 3, and 4. No. 5 received 0.25 cubic centimeter pure blood. Nos. 6 and 7 received 0.35 and 0.4, respectively, of pure blood. All the injections were made intraperitoneally.

Results (twenty-four hours after injection):
Rat No. 1, 3 spirilla found in twenty minutes' search.
Rat No. 2, none seen in forty minutes' search.
Rat No. 3, none seen in thirty minutes' search.
Rat No. 4, 4 spirilla seen in twenty minutes' search.
Mouse No. 5, 25 seen in five minutes' search.
Rat No. 6 (first control), 6 spirilla seen in fifteen minutes' search.
Rat No. 7 (second control), 7 spirilla seen in five minutes' search.
Monkey, no spirilla seen.

Results (forty-eight hours after injection):
Rat No. 1, none in twenty minutes' search.
Rat No. 2, none in fifteen minutes' search.
Rat No. 3, none in fifteen minutes' search.
Rat No. 4, none in ten minutes' search.
Mouse No. 5, 25 seen in ten minutes' search.
Rat No. 6, none in ten minutes' search.
Rat No. 7, 8 seen in fifteen minutes' search.
Monkey, 1 spirillum seen to a field.

Results (seventy-two hours after injection):
Blood of rodents all negative after ten, fifteen, or twenty minutes' search.
Monkey, 3 or 4 spirilla seen to a field. Animal went through a typical attack of the disease.
On the two subsequent days no spirilla were found in any of the rodents, and no spirilla have since reappeared.

These experiments seem to show that Bombay spirillum fever is distinct from African tick fever, but that it constitutes a form of relapsing fever very closely related, if not identical, with the forms of relapsing fever encountered in Europe and the United States. If anyone wishes to repeat these experiments I must warn him that he will be unable to obtain any white mice after leaving Europe. Neither in the laboratories of Africa nor of India was I able to obtain these animals.

Finally, from a consideration of the work performed by other investigators and from my own experiments, carried on with all these different strains of spirochætæ, including a study of the morphological characteristics, serum reactions, and animal inoculations, it appears to me that the African and European strains of spirochætæ are distinct species. However, it does not yet seem clearly demonstrated that the American and Indian strains are distinct from the European; if not identical, these strains must certainly be very closely related to one another.

DIET AND NUTRITION OF THE FILIPINO PEOPLE.[1]

By Hans Aron.

(*From the Department of Physiology, Philippine Medical School, Manila, P. I.*)

A study of the food and nutrition of a people is of great importance, both from a hygienic standpoint and from that of the intelligent practice of medicine. On the one hand, a large number of those microörganisms which we recognize as the cause of different diseases are introduced into the human body with the food. On the other hand, the quality and quantity of the food consumed is the fundamental factor in the maintenance of a normal and healthy condition of the body. It is the latter phase of the question which will receive consideration in this paper.

The study of the nutrition of a tropical people has an especial scientific interest, because our knowledge of this subject is quite limited.

The first point to be considered is the nutritive value of the food measured by its content in proteins and fuel substances, such as carbohydrates and fats. From the fact that a great part of the ingested food is burned, in order to maintain the normal temperature of the body, it has been argued that the number of calories needed by the body in a hot climate is less than the quantity required by the same body in a cold climate. This statement has met with widespread acceptance and has even found its way into scientific papers. I believe it is possible to show that this is not in accordance with the facts.

There are two main factors which regulate the heat of the human body—the one is the production of heat by combustion of organic material; the other is the loss of heat which takes place, either by conduction or radiation of heat from the surface of the body or by evaporation of water from the lungs and skin. Of minor importance is the warmth of the ingested food or the inspired air. The lower the temperature of the atmosphere, the greater is the relative amount of heat lost by conduction and radiation. Above 36° to 37° C. no heat can be lost in this way, and only water evaporation can lower the body

[1] Read at the Sixth Annual Meeting of the Philippine Islands Medical Association, February 12, 1909.

temperature. The whole heat regulation consists therefore of a balance between the production of heat by the chemical process of combustion (the chemical heat regulation) and the loss of heat by physical means (the physical heat regulation).

It has been known for a long time that in a cold climate there is an increased combustion of food stuffs, and an accelerated metabolism in general. This increase of combustion which corresponds to the burning of more coal in the furnace on a cold day, can be demostrated by estimating the amount of carbon-dioxide expired by an animal under the same conditions at different temperatures. Rubner obtained the following values in such an experiment on a guinea pig:

Temperature of air.	CO_2 in 1 hour, per kilogram of animal.
°C.	*Grams.*
11	2.15
21	1.77
26	1.54
30	1.32
35	1.27
40	1.45

While these results are unquestionably correct, it would be wrong to apply them to man for the following reason: A person living in an atmospheric temperature below 30° to 35° C., by suitable clothing protects the surface of the body against the loss of heat by conduction or radiation. Since air is a very bad conductor of heat, a layer of stationary air protects the body against loss of heat, even if the surrounding atmosphere has a lower temperature. Rubner has shown this very clearly by an experiment performed on a dog. The animal with its normal coat of hair was first kept at different temperatures and its heat production estimated, then it was shaved and the heat production again determined by the same method.

The heat production calculated per kilogram of body weight was as follows:

Temperature of air.	Dog with normal coat of hair.	Dog shaved.
°C.	*Calories.*	*Calories.*
20	55.9	82.3
25	54.2	61.2
30	56.2	52.0

This table shows that the dog with its normal coat of hair requires no additional food to maintain its normal body temperature when the atmospheric temperature is lowered from 30° to 20° C. A layer of fat has about the same protective influence as a layer of hair. This has been shown by an experiment similar to the one just described. These facts which have been demonstrated experimentally for the dog are even more strikingly true in the case of man. In civilized countries, man endeavors to render the chemical regulation of body temperature unnecessary by covering the skin with clothing, the cooler the climate, the thicker the clothes worn. Air is, moreover, the most efficient and important constituent of clothing. Fine furs are warm because they contain 98 per cent of air, which is a much poorer conductor of heat than fiber. Rubner has, furthermore, shown that a man feels comfortably warm only when the chemical regulation is completely eliminated; if this is not the case, he has a chilly feeling. Now it is clear that man in different climates will not require different quantities of fuel material to maintain his normal body temperature. As a matter of fact, by means of variations in the amount and the character of clothing, we live in all climates under about the same conditions with regard to our chemical heat regulation; and only under the supposition that we wore the light clothing of the tropics in cooler climates, would the hypothesis mentioned in the beginning of this discussion be correct. As Rubner has expressed it, man in the temperate zone is in a tropical climate as regards his heat regulation. Furthermore, we should not forget the importance of adipose tissue as a factor in heat regulation. I agree fully with Graham Lusk when he says "there can be no doubt that climatic conditions modify racial characteristics. The emigrant from northern Europe, living upon a farm in a hot and often moist climate of an American summer, must restrict his layer of adipose tissue if he is to live comfortably. The same holds true in Italy. On the contrary, the Eskimo cultivates a thick fat layer to protect himself from frost." In the Filipino there is, as a rule, an almost complete absence of the fat layer. These considerations and the conclusion that the demand for food of a civilized man in a temperate climate is not higher than in a tropical climate have been verified by the extended researches of Ejkman,[2] who has applied the method of Zuntz to determine the quantity of oxygen inspired and carbon-dioxide expired. He determined the consumption of oxygen per minute and found:

	Oxygen consumed per minute (cc.)
In Batavia, in Malays (average)	251.3[a]
In Batavia, in Europeans (average)	245.7[a]
In Europe (cold weather), in Europeans (average)	250.3[a]

[a] These figures are calculated for a body weight of 64 kilograms.

[2] *Arch. f. die gsmte. Physiol. des Mensch. u. d. Thiere.* (1896), 64, 57–78.

The best method of determining the diet of a people is to observe how much and what kind of food they consume when they choose their food according to their usual custom. It is well known that such researches were very carefully performed, first by C. v. Voit in Germany, and afterwards by many other investigators, especially by Atwater and his collaborators in America. This method is beset with great difficulties and there is the possibility of error, even if the subject is an intelligent individual. A second method consists in investigating the composition of rations dealt out to groups of individuals who have no choice as to their food, the quantity and quality of the food selected in this case being determined by the custom of the people.

By controlling the food given to soldiers, prisoners, patients in hospitals, and inmates of various other institutions, the normal diet of the average man can be determined. The following table shows the standard values of normal diets determined in this manner and for comparison those I have obtained for Filipino prisoners in Bilibid Prison in Manila.

TABLE I.

	Protein.	Fat.	Carbohydrates.	Calories.
	Grams.	*Grams.*	*Grams.*	
For a man performing medium work (according to Voit)	118	56	500	3,055
For a man performing medium work (according to Atwater)	125	Not fixed.		3,400
German prisoners (average)	107	26	550	2,959
Filipino prisoners	75	27	510	2,647

Through the courtesy of Mr. Wolfe, Director of Prisons, I have obtained an accurate list of the food-stuffs purchased for a hundred Asiatic and Filipino prisoners for the different days of the week. I have calculated, according to the average composition of the same food-stuffs as given in standard works, the content in protein, fat, and carbohydrate of this diet where there was no reason to assume a variation from this average. In other instances, where the products, such as bread, fish, and native plants, are peculiar to the Philippines, I have made some determinations myself. I am all the more inclined to believe that this method gives sufficiently accurate average values, because the composition of the food-stuffs purchased in the different months also varies; therefore it may even be more exact to regard *average* values for the composition of the food than the values found in one or two samples determined from the food-stuffs directly. The values calculated as described above are given in the accompanying table, the rations being arranged according to their protein content.

TABLE II.

	Protein.	Fat.	Carbo-hydrates.	Total calories.
	Grams.	*Grams.*	*Grams.*	
Sunday	50	47	463	2,315
Monday	60	28	521	2,640
Saturday	69	46	504	2,773
Tuesday or Friday	74	19	465	2,385
Thursday	82	18	533	2,686
Tuesday	84	23	458	2,436
Friday	89	18	571	2,872
Wednesday	96	21	572	2,934
Average	75	27	510	2,646

We see that the food given daily contains on an average about 75 grams protein, this protein content varying from about 50 to 96 grams. The caloric value of the food is about 2,650 calories and varies between 2,315 and 2,934 calories. In Table III is given the composition of the food considered from a physiological standpoint.

TABLE III.

Each prisoner receives daily:

270 grams rice 45 grams sugar 300 grams bread about 250 grams camotes or potatoes 50 to 100 grams onions	Representing about 45 grams protein and 2,100 calories.

In addition the following articles are given to each prisoner on the different days:

Sunday:	70 grams bacon,
Monday:	90 grams pork,
Saturday:	45 grams bacon and 90 grams beef,
Tuesday or Friday:	75 grams salmon, or 115 grams fresh fish,
Thursday:	100 grams corned beef and 45 grams mongo,
Tuesday:	115 grams beef and 90 grams dry fish,
Friday:	150 grams salmon and 90 grams mongo,
Wednesday:	115 grams beef and 150 grams mongo,
Daily:	3 grams tea, 5 grams coffee, 6 grams ginger root.

We see that for every day of the week, a vegetable diet, consisting of rice, sugar, bread, potatoes, and onions constitutes by far the greater portion of the nutriment furnished. This food alone, which is about the same for the different days, furnishes more than four-fifths of the calories of the entire food ingested and a great deal of the protein. In

addition to the vegetable diet, the people receive some animal foodstuffs, which show variations on the different days both in regard to their protein content and in their calorie value. For this reason the variations seen in the first table occur. We see from Table III that on certain days of the week, besides the animal food, a native vegetable called *mongo* is served as food. This vegetable, according to our analysis, is very rich in nitrogen. A very small amount of stimulants, tea, coffee or ginger root, is also given to the prisoners.

If we would compare these data with the standards given above for Europeans, we must consider that the Filipino is of considerably smaller stature than the European. While the latter has an average weight of 65 kilos, the Filipino weighs only about 50 to 55 kilos. This means that the standard value of protein determined for Europeans or Americans would have to be reduced by about 20 per cent when applied to Filipinos. The caloric requirements of a living body depend not upon its weight, but upon the extent of its surface. Now approximately the surface decreases only with the second power, while the weight decreases with the third power; in addition, I believe we should also consider that the Filipinos are thinner and taller than the European of the same weight. These considerations render it probable that the requirements in calories for Filipinos may be only about 10 per cent less than those of the European standard. If we now compare the Filipino food with that given to a European under similar conditions, we find that the calorie value of the prisoners' food corresponds to that of a workman in Europe or America, performing moderately hard labor, and also to the caloric value given in the average German prison. We have here a practical confirmation of the introductory theoretical remarks concerning the amount of calories required in the Tropics. The protein content of the food seems, even if we make a reduction of 20 per cent from the standard values, somewhat lower than that of the average European diet. Our ideas in regard to the amount of protein required by a healthy individual have recently undergone considerable changes. After Voit had given his standards for protein, some investigators showed that many people do not ingest the quantity this investigator thought necessary. At the same time the physiological question concerning the minimum quantity of protein upon which a man is able to live has been extensively investigated. It is a fact that much less protein in the food than was determined by Voit is sufficient to maintain life and health, and the values given as necessary by Chittenden, who has done the most extensive work in this direction, are considerably lower than the protein intake of the Filipinos. Therefore, even if we regard the protein quantity of the Filipino food as low, nevertheless, it is certainly sufficient. People living on an almost pure vegetable diet always take a smaller amount of protein than do meat eaters. The quantity of protein, for instance, taken

by the lower caste Bengalese in India, according to a recent research by McCay from the Medical College in Calcutta,[3] is only 30 to 40 grams. I believe that this fact depends on the wholly vegetable diet partaken of and not upon the tropical climate. One often finds in the literature the statement that the amount of protein needed in the tropics is lower than that required in a temperate climate; furthermore, because the natives eat much less protein, it is assumed that it would be unhealthful for a European to take the same amount of protein as at home. I have attempted to show that this doctrine is incorrect.

The next question to be answered is whether or not the rations issued at Bilibid Prison are a fair sample of an average Filipino diet. As already mentioned, it is very hard to answer such a question accurately, even when dealing with educated people. Therefore we will have to be content with roughly approximate values. Our most reliable method is to study the protein metabolism. The nitrogen in the urine is a measure of the protein bodies burned by the subject and if we choose for the experiment an adult man, who does his usual work, and eats his accustomed food, we can with great probability assume that the nitrogen of the protein of his food, so far as the protein is digestible, appears in the urine.[4] My student-assistant, Mr. Santos, and myself have examined the total nitrogen excreted in twenty-four hours in at least three different samples of urine from our Filipino laboratory servants. In the examinations made up to this time, we have never obtained less than 10 grams of nitrogen in twenty-four hours, and usually we have found about 12 grams, which corresponds to about 70 to 75 grams of absorbed protein. Some nitrogen determinations which were prepared on the urines of Filipino students showed a nitrogen content of about 12 to 15 grams, corresponding to from 70 to 100 grams protein. The results of these examinations warrant the statement that the quantity of protein found on an average in the prisoners' food corresponds to the protein intake of the average Filipino workman.

Concerning the estimation of the caloric value of the food of the people, we are forced to apply a rougher method. The Filipino is accustomed to take his food, together with others, from the same dishes and is hence unable to state with accuracy the quantity of food that he individually consumes. We know that the Filipino lives principally on rice and fish, some vegetables and fruits, and very seldom eats meat for the reason that it is not always, for him, obtainable. According to observations on my house servants and from information obtained by questioning my students, I have found that the amount taken is from 650 to 700 grams of rice per day and about 200 to 250 grams of fish.

[3] *Sci. Mem. Off. Med. San. Dept. India*, Calcutta, (1908) 34, 1.

[4] A part of the nitrogen is excreted in the sweat.

Such an amount of rice may be purchased for 10 centavos and the fish for 7 centavos. Such a ration would furnish about 70 to 75 grams protein, 10 grams fat and 525 grams of carbohydrates. The caloric value and also the protein value of the vegetables and fruit eaten occasionally may be neglected in such a rough calculation as this. The ration just given, of 70 to 75 grams protein, 10 grams fat, and 525 grams carbohydrates, corresponds very well in its composition and in its caloric value of 2,500 to 2,600 calories with the food issued in Bilibid.

I would like to direct your attention to still another point. Not all Filipinos, especially in the provinces and even in towns, are able to purchase regularly such a quantity of fish as I have mentioned. What would be the result if a man should omit the fish and live entirely on rice, fruits and vegetables? With the fish only a small amount of calories are ingested, chiefly proteins. The caloric value of 250 grams fish would be replaced by 60 grams rice, containing only 4 grams protein, so that the man eating only rice receives with about 2,600 calories only 50 grams protein at the most. If he wished to take the quantity of protein contained in the mixed food, in the form of rice he would have to take an immense excess of carbohydrates. This may account for the idea that rice is heating, a statement made to me by a Spanish-Filipino physician. Furthermore, vegetable proteins are not so completely digestible as animal proteins, 85 to 90 per cent of the former being digested as compared with 96 per cent of the latter.

One other point must not be forgotten. The recent researches on the chemistry of protein bodies on the one hand, and the biological reaction on the other hand, show that the question as to what constituents make up the albuminous substances may be of great importance for their value in nutrition. While it is certain that a man may continue in good health for a long time on a carefully selected purely vegetable diet, nevertheless we see that it is very often impossible to properly nourish young animals exclusively on one kind of vegetable protein. I have made such experiments on rabbits fed with corn. This has been attributed to a want of certain constitutents in vegetable proteins. Finally, I will remind you that wherever the people live exclusively on one single kind of vegetable protein, we find the appearance of certain diseases which probably have some connection with this food. I am thinking of the association of beriberi and rice, of corn and pellagra, and perhaps of the so-called scurvy of sailing vessels. I have by no means exhausted my theme, since there are many other interesting problems concerning the diet and nutrition of the Filipino people, some of which I hope to solve during my stay in these Islands.

POISONOUS SNAKES OF THE PHILIPPINE ISLANDS.[1]

By Lawrence E. Griffin.
(*From the Biological Laboratory, Bureau of Science, Manila, P. I.*)

Nearly seventy species of snakes have been described as occurring in the Philippine Islands, of which thirty species, at least, are poisonous. In view of the large number of species known, it is a matter of some surprise that snakes are so seldom encountered by those whose business leads them into the forest or through the high grass; in fact, the majority of people seem to believe that very few snakes exist here. The finding of two new species in the small collection of the Biological Laboratory, Bureau of Science, leads me to believe that when our Philippine snakes have been carefully collected and studied, a considerable number of species will be added to the herpetological fauna of the Islands.[2] As the technical description of these two snakes is uninteresting and is to appear in Section A of the Journal,[3] I beg your permission to digress from this subject to that of Philippine poisonous snakes in general.

Of most general interest is the snake known as the rice-snake, or *"dahun-palay"* (*Dryophis prasinus*), of which the natives stand in such fear. An extremely slender snake, generally bright green in color, it is supposed to live among the rice stalks. As a matter of fact, while it may be found occasionally in the rice, it is really a tree snake, living often in the tops of the coconuts, or branches of forest trees. Its bite is supposed to be fatal, death ensuing in from fifteen minutes to half an hour. Many natives believe that the leaves wither upon which its breath has fallen. While undoubtedly poisonous, this snake is one of those in which the fangs are at the back end of the maxilla, so far back that the snake would have to stretch its mouth tremendously to bite an object the size of a man's leg. Information as to deaths proven to have been caused by the bite of this snake will be appreciated.[4]

[1] Read at the Sixth Annual Meeting of the Philippine Islands Medical Association, February 13, 1909.

[2] Since reading this paper, there have been found in collections of snakes from Palawan, P. I., alone, four new species, and three species not hitherto recorded from the Philippines.

[3] *This Journal, Sec. A* (1909), 4, 55.

[4] The Director of Health has kindly sent me a copy of a letter relating to two deaths from snake bite, supposed to have been caused by the *Dahun-palay*. In neither instance was the snake seen, while the nature and place of the attacks leaves the possibility open that cobras were responsible for both deaths.

The question is often asked, "are there any cobras in the Philippines?" We have three species of cobras in our collection. First, the hooded cobra, the cobra de capella, *Naja naja*, one fully grown female specimen of which has been caught within 4 miles of Manila. There seems to be good reason to believe that cobras are much more plentiful in the Islands than is supposed, and that many of the deaths from snake bite are to be laid to them. A nearly black variety, which may prove to be a distinct species, is found in Palawan. Secondly, a large specimen of the king cobra, *Naja bungarus*, measuring more than 8 feet in length was caught in Benguet, and is now in the collection of the Bureau of Science. In spite of its size and venom, this species probably lives to our benefit rather than harm, for it is said to feed on nothing but other snakes. Finally, in Samar, Leyte, and in Mindanao, is a species of cobra found only in the Philippines, *Naja samarensis*. If, as I believe, certain reports of a snake in Samar, which have come to me lately, refer to this species, it is as active a pest as its relative in India.

Probably the most vicious appearing snake in the Philippines is the bamboo snake, *Trimeresurus gramineus*. This snake is found in clumps of bamboo, or hanging from the limbs of trees by its short prehensile tail. The general color of the body is bright green, while the tail is red. It is armed with fangs four times as large in proportion to its size as those of the cobra, though it is doubtful if its venom is as deadly as that of the cobra. This snake is fairly common, and widely distributed. In the southern Islands there are found at least three more species of the same genus. In China this species is considered very dangerous on account of its habit of hanging suspended by its tail from branches, and striking when disturbed.

The other poisonous land snakes of the Islands are mostly of small size. A few species are greatly feared, but most of them are too small to do much damage to human beings. The snakes of the genus *Doliophis* are interesting because of the enormous development of the poison glands, which occupy a third the length of the body, and which, by their extension backward, have crowded the heart some distance posterior to its usual position. There is on the part of the Filipinos a great deal of fear and superstition regarding another snake, the tiny *Typhlops braminus*, which, when full grown, is no larger than a small earthworm. It is found very often in termite nests, without regard to whether or not these are occupied. The most usual native superstition regarding this snake is that if it bites a carabao the latter will die immediately. Inasmuch as the mouth of the *Typhlops* would scarcely admit a single hair of the carabao, and microscopic teeth are borne only by the maxilla, one is at a loss to find the basis for this superstitious belief.

REVIEWS.

A Text-Book of General Bacteriology. By Edwin O. Jordan, Ph. D. Pp. 557. Price $3.00 net. Philadelphia and London: W. B. Saunders Company, 1908.

Professor Jordan in his General Bacteriology has not only presented much of his material from new points of view, but he has arranged it to the best advantage. This is especially true of his treatment of the very important group of colon-typhoid organisms. The chapter on immunity is short but it includes the essential points and these are given in a clear, concise manner. The illustrations are exceptionally good and add much to the value of the book. The language is clear and the subject-matter is treated in an easy, attractive style. It would seem that the field of protozoölogy has assumed such proportions that it should be considered in a separate book and not in such a work on bacteriology, since it forms no true part of this subject. However, Professor Jordan's chapter on the protozoa does not detract from the value of the book. The closing chapters upon the higher organisms, the bacteriology of milk and milk products, the nitrogen cycle, etc., serve to enhance its value as a text-book. The author has stated facts as facts and where differences of opinion prevail has given both sides impartially, yet he does not hesitate to express his own opinions. The bibliography is not extensive but is ample, and includes the most recent and most important references. In my opinion it is the best text-book in English on bacteriology and it will be used in the Philippine Medical School.

The topography and general make-up of the book are in keeping with its contents, and both author and publisher are to be congratulated on the result of their work. The book will be valuable not only to the student of medicine but to the general practitioner and to the advanced bacteriologist.

V. L. Andrews.

Diseases of the Skin and the Eruptive Fevers. By Jay Frank Schamberg, A. B., M. D. Pp. 534. Price $3.00 net. Philadelphia and London: W. B. Saunders Company, 1908.

Doctor Schamberg devotes 380 pages of this volume to diseases of the skin and the rest of his space to the eruptive fevers. While the discussion of the various skin diseases is necessarily brief, it is clear and practical. The discussion of the eruptive fevers is confined mainly to the skin manifestations of these diseases. Yaws is given one page, while syphilis is given twenty-seven pages. *Treponema pallidula* is not mentioned in

connection with yaws, though *T. pallida* is given as the probable cause of syphilis. It is not believed that the simple statement that mild cases of yaws "yield readily to mild parasiticides," and that tonics are required for the severe cases, fairly states the requirements or the present practice in the treatment of the disease. Oriental sore is not mentioned. Blastomycosis cutis is described twice. The articles on actinotherapy and radiotherapy, radium, and serum eruptions are concise and to the point.

The numerous illustrations are excellent. The book will be a welcome addition to the library of the general practitioner.

E. R. WHITMORE.